Universität Rostock Traditio et Innovatio

Aus der Professur für Landschaftsökologie und
Standortkunde der Agrar- und
Umweltwissenschaftlichen Fakultät

Short-term biogeochemical effects of agricultural management measures in soils amended with anaerobic biogas digestate

Kumulative Dissertation

zur Erlangung des akademischen Grades
Doktor der Agrarwissenschaften (doctor agriculturae)

an der Agrar- und Umweltwissenschaftlichen Fakultät
der Universität Rostock

vorgelegt von M.Sc. Sebastian Rainer Fiedler
Rostock

Rostock, den 29. September 2017

urn:nbn:de:gbv:28-diss2018-0139-3

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Date of defence

15 June 2018

Danksagung

Ich möchte mich für die Unterstützung und Inspiration all jener Bedanken, die mich auf meinem bisherigen Weg begleitet haben und mir die Möglichkeiten gaben, mein Leben, so wie es ist, führen zu können. Damit meine ich von Seiten meiner Familie in erster Linie meine Eltern, Cornelia und Klaus-Rainer, sowie meine liebe Schwester, Anna. Von aka-demischer Seite danke ich zuvörderst Stephan Glatzel, der mich mit diesem spannenden Themenkomplex überhaupt erst in Berühung und mich dadurch mit den wunderbaren Menschen seiner Rostocker Arbeitsgruppe in Verbindung brachte. Zu diesen gehört der vor Ideen sprühende Gerald Jurasinski, Anke Günther, Franziska Koebsch, Vytas Huth und Marian Koch, die mir alle bei meinen Fragen und Problemen – u. a. beim Program-mieren mit R – geduldig weiterhalfen. Karin Jagusch und Birgit Schröder, haben mir mit ihrem Durchblick in administrativen Angelegenheiten überhaupt erst einen flüssigen Ab-lauf des Alltags ermöglicht. Bedanken möchte ich mich auch bei Stefan Köhler, der häu-fig genug ein Auge zudrückte, wenn ich in seinem Reich agierte. Sehr viel Arbeit haben mir auch die unzähligen Studenten unter Anleitung der technischen Angestellten Sascha Tittmar und Steffen Kaufmane während der Feldmessungen abgenommen, ohne deren unermüdlichen Einsätzen zu zum Teil „unchristlichen" Zeiten gar keine Datengrundlage vorhanden gewesen wäre. Im Rahmen des Forschungsprojektes danke ich Jürgen Augus-tin, der mehr oder weniger als „Drahtzieher" im Hintergrund der geistige Vater der vor-liegenden Dissertation ist, sowie Ulrike Hagemann, welche mit einer beachtenswerten Effizienz die einzelnen Arbeitsgruppen koordinierte. Ganz besonderer Dank gilt Nicole Wrage-Mönnig, die mich mit einer unfassbaren Geduld zum Abschluss kommen ließ –ich wünsche und nehme mir vor mich dafür in den nächsten Monaten mit den entspre-chenden Ergebnissen zu revanchieren. Ein Dankeschön geht von mir auch an Jana Peters und Andreas Gurgel von der Landesforschungsanstalt für Landwirtschaft u. Fischerei Mecklenburg-Vorpommern in Gülzow für die gute Zusammenarbeit auf deren Versuchs-flächen. Zum Abschluss danke ich auch dem sogenannten Steuerzahler und damit implizit auch all jenen, deren Arbeitskraft – freiwillig und unfreiwillig – weltweit verwertet wurde, um mir diese Dissertation überhaupt erst zu ermöglichen.

Kurzfassung

Die Nutzung von Biogas als Energieträger kann fossile Brennstoffe ersetzen und so zu einer Reduktion von Treibhausgasemissionen beitragen. Die im Biogasreaktor entstehenden Gärreste (GR) werden ferner als organischer Dünger genutzt. Im Vergleich zu unvergärtem Substrat haben GR einen verringerten Gehalt an organischen Kohlenstoff (C), einen erhöhten Anteil an Ammoniumstickstoff (NH_4^+-N) sowie einen erhöhten pH-Wert. Diese Eigenschaften könnten sich negativ auf die Emissionen von Distickstoffmonoxid („Lachgas“ bzw. N_2O, ein potentes Treibhausgas) aus landwirtschaftlich genutzten Böden sowie auf die Bildung organischer Bodensubstanz (OBS) und somit auf die Speicherung von C sowie die Bodenfruchtbarkeit in den betroffenen Böden auswirken.
Bodenbearbeitungsmaßnahmen wie etwa Pflügen, Grubbern und Eggen befördern unmittelbar die mikrobielle Zersetzung der OBS durch die Zerkleinerung schützender Makroaggregate, was die heterotrophe Bodenatmung (R_h) erhöht. Dies führt unter anderem dazu, dass die temperaturabhängige Modellierung der R_h gestört wird und neu validiert werden muss. Bisher war es allerdings unklar, wie stark und wie lange diese Störung anhält und welchen Effekt die Nutzung unterschiedlicher Düngemittel möglicherweise hat. Es war ebenfalls unbekannt, wie Bodenbearbeitung die Zersetzung von OBS auf molekularer Ebene beeinflusst, insbesondere bei gleichzeitiger Nutzung von GR.
Das Ausbringen N-haltigen Düngers ist in der Regel mit temporär hohen N_2O-Emissionen verbunden. Zusätzlich sind GR besonders anfällig für N-Verluste in Form von Ammoniak (NH_3). Um diese Verluste zu reduzieren, empfiehlt sich nach der „guten fachlichen Praxis” die Injektion des GR in den Boden. Gleichzeitig kann diese Maßnahme jedoch die Emission von N_2O befördern, was im Vergleich zu einer Einarbeitung des GR in die Bodenoberfläche möglicherweise die unterbundenen NH_3-Verluste sowohl in der N- als auch in der Umweltbilanz nivellieren würde. Des Weiteren könnte die Emission von molekularen Stickstoff (N_2) einen signifikanten Beitrag zu den gasförmigen N-Verlusten leisten, was aber aus methodischen Gründen nur wenig erforscht ist.
Diese Dissertation untersucht daher mit besonderem Schwerpunkt auf GR die Fragestellungen: (*i*) Wie wird die temperaturabhängige Modellierung der R_h durch Bodenbearbei-

tung im Allgemeinen beeinflusst und welche Auswirkungen hat die Nutzung unterschiedlicher Düngestrategien im Besonderen? (*ii*) Welche umgehenden Veränderungen der OBS verursacht Bodenbearbeitung und welche Bestandteile sind davon betroffen? (*iii*) Wie werden die N_2O- und N_2-Emissionen nach der Applikation von hohen GR-Konzentration (wie sie nach der Injektion in Böden auftreten können) durch Bodentyp, -Wassergehalt und der GR-Menge beeinflusst?

Die mit GR gedüngten Prüfglieder zeigten in Bezug auf Kohlendioxid (CO_2) ein von den anderen, d.h. ungedüngten bzw. mineralisch gedüngten, Prüfgliedern abweichendes Emissionsverhalten, denn die durch die Bodenbearbeitung geförderten Mineralisationsprozesse führten in der GR-Variante zu einem wesentlich niedrigeren Anstieg der R_h. Dieser gehemmte Anstieg der R_h nach Bodenbearbeitung ist wahrscheinlich auf die geringere Verfügbarkeit von leicht zersetzbaren C-Verbindungen im GR-Dünger zurückzuführen. Möglicherweise hat die Freisetzung antimikrobielle Stoffe wie Sterole, die sich in GR finden lassen, ebenfalls zu einem geringeren Anstieg der Aktivität des Bodenlebens beigetragen. Das abweichende Emissionsverhalten ließ sich auch anhand einer schwächer und kürzer ausgeprägten Störung der temperaturabhängigen R_h-Modelle erkennen. Dies verdeutlichte sich im Vergleich zu den anderen Düngestrategien an einer geringeren Streuung des Aktivierungsenergieparameters E_0 sowie an einer sich nach weniger Tagen erholenden Modellgüte.

Die vermuteten Änderungen der OBS-Zusammensetzung infolge einer Düngung mit GR ließen sich mittels Pyrolyse-Feldionisationsmassenspektrometrie bereits nach einer Anbausaison mit Mais (*Zea mays* L.) nachweisen. Die Düngung mit GR führte im Vergleich zur mineralischen Düngung zu einer Abnahme des Anteils leicht zersetztbarer Bestandteile wie Kohlenhydrate, während sich der Anteil schwer zersetzbarer Bestandteile wie Lignin erhöhte. Jedoch wurden Hinweise auf eine relativ kurzfristige Zersetzung ligninhaltiger Stoffe gefunden, was die angenommene schwere Zersetzbarkeit dieser den Gärprozess überstehenden Substanzen infrage stellt. Eine mögliche Erklärung dafür ist, dass das durch die Bodenbearbeitung aktivierte mikrobielle Bodenleben wegen des Mangels an leicht verfügbaren C-Verbindungen auf diese ligninhaltigen Stoffe zurückgreift. Davon unabhängig konnte aber der verstärkte Abbau von Kohlenhydraten in der mineralisch Gedüngten Variante bestätigt werden.

Die Anwendung hoher GR-Konzentrationen, wie sie bei der Düngung mittels Injektion auftreten können, ergab, dass die anschließenden Emissionen von N_2O und N_2 insbesondere vom Gasdiffusionsvermögen des Bodens als Funktion seiner Textur und seines Wassergehalts abhängen. Der feinkörnige, tonige Schluff emittierte aufgrund seines schlechteren Diffusionsvermögens höhere Flussraten und zeigte eine stärkere Interaktion mit dem Wassergehalt als der grobkörnige, lehmige Sand, d.h. das N_2 / (N_2O+N_2)-Verhältnis der Flussraten erhöhte sich mit steigendem wassergefülltem Porenvolumen. Eine Verdopplung der GR-Düngemenge verursachte keine signifikante Änderung der N_2O- und N_2-Flussraten, wahrscheinlich wegen der hemmenden Wirkung hoher NH_3- bzw. NH_4^+-Konzentrationen auf Nitritoxidierer. Dies könnte in Verbindung mit dem erhöhten pH-Wert des GR zu einer Anreicherung von Nitrit (NO_2^-) geführt haben, welches zur Entgiftung wiederum in Stickstoffmonoxid (NO), N_2O und N_2 reduziert worden wäre. Die gemessenen N_2O- und N_2-Flussraten in Verbindung mit den vermuteten NO-Emissionen infolge der hohen GR-Konzentration stellen den postulierten positiven Umwelteffekt der Injektionsmethode in Frage.

Ingesamt lässt sich feststellen, dass der mit GR gedüngte Boden eine geringere Affinität für C-Verluste nach Bodenbearbeitung aufwies. Dieser Nachweis stützt die Annahme, dass der Einsatz von GR keine negativen Effekte auf die OBS ausübt, da die leicht zersetzbaren C-Fraktionen, die während des Gärprozesses mikrobiell umgesetzt werden, vermutlich die gleichen sind, die nach Bodenbearbeitungsmaßnahmen mineralisiert worden wären. Gleichzeitig wurden jedoch Hinweise gefunden, dass die “gute fachliche Praxis” der Injektion flüssiger, organischer Dünger in Bezug auf GR mit seiner veränderten Zusammensetzung möglicherweise angepasst werden muss, um den gasförmigen N-Verlust mit seinen negativen Umweltwirkungen zu minimieren. Daher ist weitere Forschung zu den Folgen des GR-Einsatzes als Dünger dringend geboten.

Abstract

The use of biogas for energy production is measure to reduce greenhouse gas (GHG) emissions by substituting fossil fuels. The residues from the anaerobic digestion in biogas plants (biogas digestate, BD) are used as amendment for agricultural soils. Biogas digestate is characterised by a lower organic arbon (C) content, an increased proportion of ammonium (NH_4^+) as well as an upward shifted pH compared to the undigested precursors. Hence, its use may have consequences for N emissions from soils, e.g. nitrous oxide (N_2O), and impacts on soil organic matter (SOM) reproduction, i.e. soil fertility and C sequestration.

Tillage enhances microbial decomposition of SOM, which is accompanied by increased soil respiration (SR) in the short-term. As one result, tillage disturbs the calibration of temperature-driven SR models. It was unclear to which extent and how long tillage will hamper re-calibrating and how the use of different fertilisers like BD would affect this disturbance. It was also unknown how tillage coincidently affects decomposition processes of SOM on the molecular level, especially after amendment with BD.

Fertiliser application generally induces a period of high N_2O emissions from agricultural soils. Compared to other fertilisers, BD is additionally loss-prone for ammonia (NH_3). To reduce these losses, best practices recommend the injection of BD into soil. However, this measure can concurrently increase N_2O emissions compared to a homogeneous surface incorporation, which may counterbalance reduced NH_3 losses in terms of nitrogen (N) savings and environmental pollution. Moreover, dinitrogen (N_2) emissions after the injection of BD may constitute a significant pathway for N losses but are scarcely examined.

Focussed on BD-treated arable soils, the present thesis examines consequently the questions: (*i*) how is the temperature-driven modelling of SR affected after tillage in general, and what are the impacts of different fertiliser treatments in particular? (*ii*) Which short-term changes in SOM does tillage induce and which compounds are affected? (*iii*) How do soil type, moisture and the amount of BD affect emissions of N_2 and N_2O from arable soils after the application of high BD concentrations comparable to injection into soils?

The treatments amended with BD revealed a distinct C dioxide (CO_2) emission pattern compared to unamended and mineral fertilised treatments during the days after tillage. The increase in SR due to tillage-induced mineralisation by macroaggregate comminution was significantly lower in BD treatments. Most probably, the restricted availability of readily biodegradable C compounds from the amendment with BD hampered the additional respiration activity after tillage. The coincidently reduction of microbial activity by anti-microbial substances like sterols could have been an additional treat to SR. The distinct behaviour of BD treatments was also reflected in a less pronounced and shorter disturbance of temperature-dependent SR models after tillage, i.e. the activation energy parameter (E_0) showed a lower variance and the model quality regained after fewer days compared to mineral fertilised treatments.

Pyrolysis-field ionisation mass spectrometry (Py-FIMS) showed that the suggested differences in SOM composition caused by the different fertilisers appeared after only one season in a maize (*Zea mays* L.) field. The amendment with BD resulted in a lower share of easily decomposable compounds like carbohydrates but a higher share of recalcitrant compounds like lignin compared to the mineral fertilised treatment. Additionally, indications for the short-term turnover of lignin-derived substances were observed during the days after tillage. This finding questions the recalcitrance of these stable leftovers from anaerobic digestion and is hypothesised to result from the scarcity of easily available C for microorganisms, which let the latter draw on lignin-containing sources instead. Nevertheless, the depletion of carbohydrates due to increased mineralisation after tillage was confirmed for the mineral fertilised treatment.

Directly after the application of BD in concentrations typical for injected application in arable soils, the emissions of N_2O and N_2 were controlled mainly by gas diffusivity as a function of soil texture and water content. Compared to the coarse textured loamy sand, the fine textured clayey silt was less favourable for gas diffusion, which resulted in higher flux rates and a more pronounced interaction with soil water, i.e. the N_2 / (N_2O+N_2) product ratio increased with water-filled pore space (WFPS). Doubling the amount of applied BD showed no significant effects on the emitted flux rates of N_2O and N_2, probably due to the inhibitory effect of high NH_3 and NH_4^+ concentrations, respectively, on nitrite oxidisers. As a result, nitrite may have accumulated and been reduced subsequently to nitric oxide (NO), N_2O and N_2 for detoxification. The measured emissions of N_2O and N_2 and

the supposed NO losses resulting from high concentrations of BD in soils question the environmental benefits of BD injection into soil compared to surface application.

Overall, the BD amended soils were less prone to C losses after tillage. This finding supports the recent indications of absent compromising effects on soil fertility after a regular application of BD since the C fractions, which were already converted into biogas during AD, would otherwise decompose preferably in soils after tillage. However, the recommendations of best practices for management activities like application by injection should be checked and adapted to the unique characteristics of BD if applicable. Further research is recommended to minimise the environmental impact of the use of BD as an organic fertiliser.

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1. General Introduction

1.1 Role of anthropogenic greenhouse gas emissions from agriculture for climate change

The composition of our atmosphere is unique in the solar system, because it is largely determined by biological processes in soils, vegetation and the oceans. However, with the industrial revolution, the biogeochemical cycling of major nutrients including nitrogen (N) and carbon (C) has changed towards a "pollution" with C dioxide (CO_2) and reactive N species like nitrous oxide (N_2O) and ammonia (NH_3) (Fowler et al., 1998; Fowler et al., 2009; Fowler et al., 2013).

Anthropogenic related increases in the concentrations of greenhouse gases (GHGs) in the atmosphere have substantially enhanced the greenhouse effect (Myhre et al., 2013), implying a potential ecological crisis if current mitigation efforts remain inadequate (Raftery et al., 2017). The increase of the atmospheric abundance of the three major GHGs reached new highs in 2015, with CO_2 at 400.0 ± 0.1 ppm (mean ± standard deviation), nitrous oxide N_2O at 328.0 ± 0.1 ppb and methane (CH_4) at 1845 ± 2 ppb (Figure 1-1). These alues exceed the pre-industrial (before 1750) levels by 44 % (CO_2), 21 % (N_2O) and 56 % (CH_4) and continued to increase by an annual absolute mean of 2.08 ppm yr^{-1} (CO_2), 0.89 ppb yr^{-1} (N_2O) and 6.0 ppb yr^{-1} (CH_4) between 2014 and 2015 (World Meteorological Organization, 2016).

Globally, agriculture accounts for estimated 5.0–5.8 Gt equivalent CO_2 yr^{-1} or about 11% of total anthropogenic GHG emissions, excluding emissions from land-use change (Smith et al., 2014). Moreover, agriculture is the largest contributor to global anthropogenic non-CO_2 GHG emissions, namely N_2O and CH_4, accounting for 84 and 52%, respectively, while agricultural soils may act as a small sink or source of CO_2 (Smith et al., 2008). N_2O and CH_4 are potent greenhouse gases with global warming potentials (integrated over 100 years, with reference to CO_2) of 298 and 34, respectively (Myhre et al., 2013). Agricultural N_2O emissions are mainly a result of mineral N fertiliser and manure application to soils (Davidson and Kanter, 2014), whereby "roughly a quarter of the annual fertilizer production [is] effectively fertilizing the atmosphere" (Fowler et al., 2015). The major CH_4 sources from this sector are ruminant animals, flooded rice fields and animal waste (Mosier et al., 1998b). However, CH_4 will not be further considered here since the focus of this thesis lies on GHG fluxes from arable non-paddy soils. Regarding the CO_2 balance

of soils, only soil organic matter (SOM) derived CO_2 contributes to changes in atmospheric CO_2 concentration (Kuzyakov, 2006). Since SOM-C exceeds the pools of C in aboveground biomass nearly three times and in atmospheric CO_2 approximately double (Eswaran et al., 1993), it is a very large potential source of CO_2 if decomposition exceeds humification (Kuzyakov, 2006). Respiration and N_2O generation are quantitatively the most important GHG sources from arable non-paddy soils and are a result of manifold biological processes (see section 1.2).

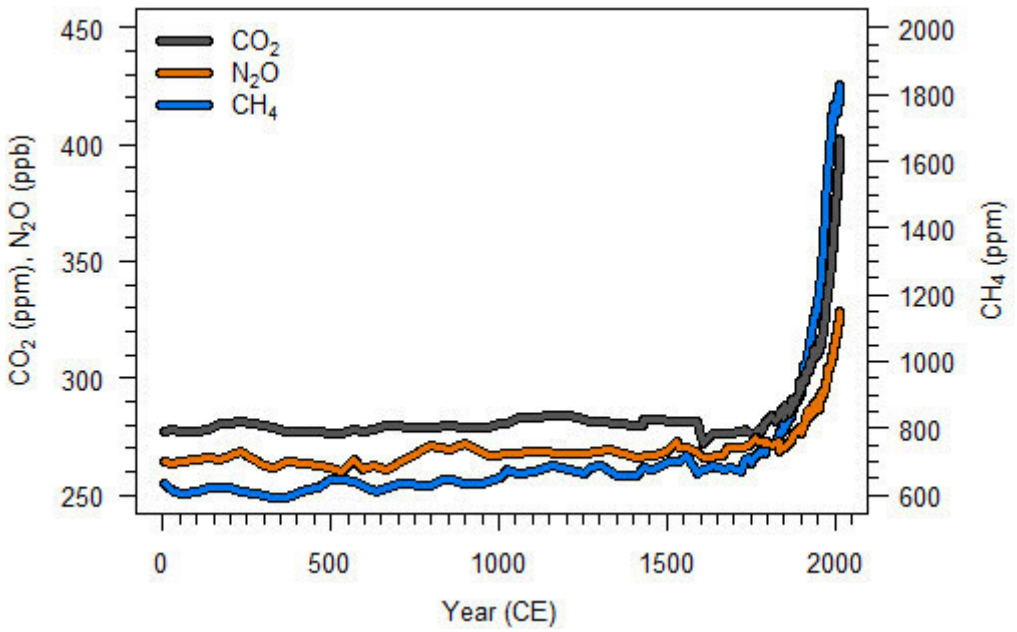

Figure 1-1. Atmospheric concentrations of important greenhouse gases from 1 to 2016 CE. Own presentation of data taken from ice core and firn air measurements from Law Dome, Antarctica, by MacFarling Meure et al. (2006) (1–1990) and from flask air samples from South Pole, Antarctica, by CSIRO Oceans and Atmosphere (2017) (1991–2016).

Due to increased food demand induced by an increasing global population, scenarios indicate that agricultural and agriculture-related emissions will become even more important in the future (Bajželj et al., 2014). Thus, agriculture is crucial to meeting global mitigation targets of holding the rise in global average temperatures by 2100 below 2 °C above pre-industrial levels and to pursue efforts to limit global warming to 1.5 °C above pre-industrial levels (Wollenberg et al., 2016). But currently, the GHG emissions from global agriculture are increasing at around 1 % per annum yet substantial cuts in emissions are needed across all sectors (Smith et al., 2014). Thus, finding mitigation strategies for emissions from agricultural soils is crucial and for this reason, a better understanding of the influencing variables of N_2O and CO_2 generation from agricultural soils is important.

Concurrently, the use of crops dedicated for energy production is postulated as a measure to reduce GHG emissions from burning of fossil fuels (Smith et al., 2014). Nevertheless, this development might foster an increase in arable land use and associated emissions – additional to the need for an increase in food production. Further, if natural vegetation is converted to annual crops, this land use change may result in high C losses (Don et al., 2012) and could completely offset potential GHG savings from substituting fossil fuels with energy from biomass farming (Whitaker et al., 2010). However, there are indications that the savings of CO_2 emissions from bioenergy use might also be offset by the N_2O emissions from the accompanied use of N fertiliser (Crutzen et al., 2008).

In Germany, the promotion of biogas plants has become a prominent regulatory action in the shift to the use of renewable energies in the last two decades (Rehl and Müller, 2011). As a result, the use of residues from anaerobic digestion (AD) in these plants (biogas digestate, BD) as amendment for agricultural soils has increased accordingly. Hence, BD is a relatively new soil amendment and almost no long-term observations are available. As a result, the consequential effects on N_2O emissions from soils and C balance in soils are still under debate. Generally, BD is characterised by an altered C and N composition as well as a shift in pH compared to the undigested precursors (Möller, 2015) (see section 1.3). This implies the continued requirement for studies about the impact of BD on gaseous N losses and SOM composition, including how soil amendment with BD alters the short-term effects of field management activities like fertiliser application and tillage (see section 1.4).

1.2 Biogeochemical processes associated with emissions of carbon dioxide and nitrous oxide from agricultural soils

1.2.1 Soil autotrophic, heterotrophic respiration and the role of C sequestration

Soil respiration (i.e. the biotic flux of CO_2 from soil to atmosphere, SR) is one of the major fluxes in the global carbon cycle and the main pathway for C moving from the ecosystem to the atmosphere (Hashimoto et al., 2015; Ryan and Law, 2005). It is highly responsive to changes in soil temperature and moisture (Raich and Potter, 1995) and hence global climate change will alter bulk SR. The first recorded measurements of carbon dioxide from soils were made in 1852 by Boussingault and Lewy (as cited in Lau, 1906). Although it has been recognised some years later as a microbial process (Schlösing, 1888), a better understanding of the processes involved is still required for an

accurate prediction of SR under future climatic conditions (Chen et al., 2011b). Soil respiration is a huge potential source of CO_2 emissions since C stored in soils worldwide exceeds the amount of C stored in phytomass and the atmosphere (Scharlemann, J. P. W. et al., 2014). The actual estimated global annual mean of S R amounts to 91 P g C yr $^{-1}$ (be-tween 1965 and 2012; Monte Carlo 95 % confidence interval: 87–95 Pg C yr^{-1}) and in-creases at a rate of 0.09 Pg C yr^{-1} (Hashimoto et al., 2015). Nevertheless, consensus is lacking about C fluxes from soils since the size of global soil organic C (SOC) stocks and their spatial distribution are uncertain due to changes in land use and land cover (Schar-lemann, J. P. W. et al., 2014).

Soil respiration is composed of heterotrophic and autotrophic respiration (Kuzyakov, 2006). Additionally, there is also a possible contribution of abiotic CO_2 release from carbonates in alkaline (pH > 7) soils (Bertrand et al., 2007). Heterotrophic respiration encompasses the microbial consumption of root exudates and the decomposition of dead roots and SOM (Smith et al., 2010). Most CO_2 evolved by heterotrophic soil organisms is emitted by microorganisms (bacteria, fungi, actinomycetes and protozoans), while the soil macrofauna contributes only a few percent. Plants with their contribution by root respiration are the most important autotrophs, whereas other autotrophs as algae and chemolitotrophs are of minor importance (Kuzyakov, 2006). Because substrate availabil-ity controls SR, plant metabolism, photosynthesis and the decomposition of plant residues exhibit a large influence on SR (Ryan and Law, 2005) (Figure 1-2). Accordingly, the leaf area index as a measure of site productivity correlates with SR. Chen et al. (2014) estimated the average annual global SR of croplands at 546 g C m^{-2} yr^{-1}, with autotrophic SR contributing 38.3 %. Autotrophic SR was found to account for 17 % of the net crop assimilation of maize (Rochette et al., 1999). However, Remus and Augustin (2016) modelled after ^{14}C pulse labelling that only 6 % of C assimilated by spring rye during a growth season where respired autotrophically and additional 3 % were detected as rhizodeposition, potentially contributing to heterotrophic SR.

Globally, heterotrophic and autotrophic SR are estimated to contribute 51 and 40 Pg C yr^{-1}, respectively (Hashimoto et al., 2015). However, only heterotrophic SR originates in SOM decomposition and, thus, controls the soil as a sink or a source of C (Kuzyakov, 2006). Nevertheless, plants as autotrophic organisms affect heterotrophic activity directly

by providing rhizodeposition of easily available C sources that may increase or decrease mineralisation of SOM, known as priming effect (Kuzyakov et al., 2000). Notably, not all CO_2 produced by respiration in soils is emitted immediately. Although in the long-term, all CO_2 produced in a soil must be emitted by the surface, CO_2 may be temporary entrapped in soil pores after precipitation or – in acidic soils – dissoluted in soil water and transformed to bicarbonate ions (Maier et al., 2011; Angert et al., 2015).

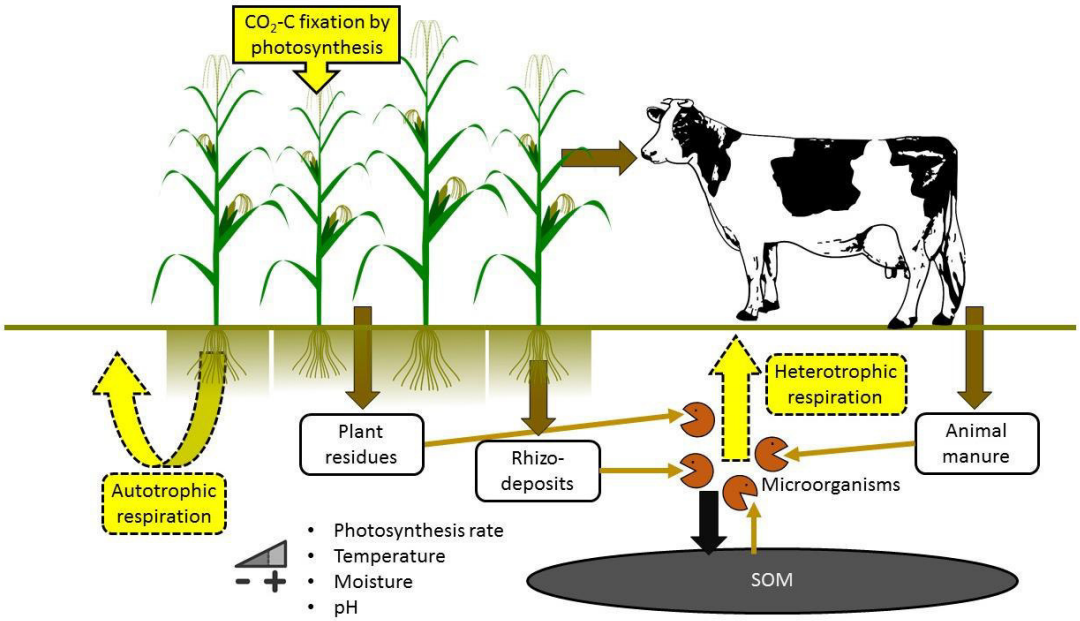

Figure 1-2. Constituents of soil respiration in an agricultural soil. Soil respiration accrues from autotrophic respiration by plant roots and heterotrophic respiration by mostly microorganisms. The latter consume atmospheric CO_2-C fixed by plants in the form of rhizodeposits and dead plant residues as well as manure from animals. Microbes also consume soil organic matter (SOM), which in turn consists to large parts of microbial residues. Own presentation.

Generally, SR features a pronounced seasonal pattern in most locations, with maximum fluxes coinciding with periods of active plant growth (Raich and Potter, 1995), when plant assimilation and hence root activity is highest (Rochette et al., 1999). The relationship between SR and temperature can be accurately described by an Arrhenius type equation (Eq.$SR = R_{\mathrm{ref}} \exp\left[E_0 \left(\frac{1}{T_{\mathrm{ref}} - T_0} - \frac{1}{T - T_0}\right)\right]$,

(1-1), which gives a decrease in effective activation energy with increasing temperatures, where the SR rate is relatively more sensitive to temperature changes at low temperatures (Lloyd and Taylor, 1994):

$$SR = R_{\mathrm{ref}} \exp\left[E_0 \left(\frac{1}{T_{\mathrm{ref}} - T_0} - \frac{1}{T - T_0}\right)\right], \quad (1\text{-}1)$$

where SR is the measured SR rate (mg CO_2-C m^{-2} h^{-1}), R_{ref} the respiration at the reference temperature (mg CO_2-C m^{-2} h^{-1}), E_0 an activation like parameter (K), T_{ref} the reference

temperature (283.15 K), T_0 the temperature constant for the start of biological processes (227.13 K), and T the temperature of soil of air (K).

Besides temperature, soil moisture is a major factor controlling SR (Raich and Schlesinger, 1992). SR decreases, on the one hand, when soil moisture becomes limited for metabolic activity and transport of nutrients to respiring organisms and, on the other hand, when high soil moisture saturates pores and hence oxygen (O_2) availability for microorganisms becomes limited (Wang et al., 2000). Further, the availability and quality of organic C also drives respiration (Tiedje, 1988; Uchida et al., 2012; Liu et al., 2006), hence the addition of organic fertilisers like manure can increase SR (Rochette and Gregorich, 1998; Ciais et al., 2010). Nan et al. (2016) observed the largest share of SR (and CO_2 efflux) occuring in (and from) the soil surface (0–15 cm) in maize fields, accounting for ca. 75 % of the total amounts in the soil profile (0–100 cm). They accounted this stratification to the effect of plant roots and an increasing soil bulk density with depth, i.e. low gas diffusion through the plough pan. In natural soils, there is also a decrease of CO_2 production with depth, which is accounted to a decreasing C and nutrient availability as well as temperature (Davidson et al., 2006; Fierer et al., 2003). Soil temperature is the dominant driver of SR in global models. However, within certain biomes (i.e., temperate evergreen forests, temperate deciduous forests and boreal forests), soil moisture and soil carbon can emerge as the dominant predictors (Hursh et al., 2017). Additionally, microbial biomass and clay content also govern heterotrophic SR (Colman and Schimel, 2013). The ability of soil microorganisms to adjust their C use efficiency (the ratio of growth over C uptake, CUE) to the C and N availability implies an effect of N deposition and fertilisation on heterotrophic SR (Manzoni et al., 2012; Eberwein et al., 2015). The hypothesis of CUE assumes that with a low availability of nutrients (e.g. N) microorganisms take up more SOM and hence respire more C in order to mine nutrients. This overflow respiration might decrease soil C storage. Conversely, an improved nutrient availability might decrease SR. Accordingly, Zhong et al. (2016) found in a meta-analysis that SR was negatively correlated with the N levels in forests and maize fields but, however, positively with the N levels in grasslands and wheat fields. Zhou et al. (2014) attribute the increase of SR following N fertilisation to an increased autotrophic SR in grasslands and croplands. Nevertheless, temperature may play a crucial role in this complex interaction in reference to SOC storage under the prospective climate change since temperature is

correlated negatively with CUE (Manzoni et al., 2012; Eberwein et al., 2015). Nevertheless, arable soils are characterised by low C content or low C / N and showed no enhanced temperature sensitivity of SR after induced warming (Karhu et al., 2014).

The bulk of the opposing C flux into soils is comprised by the indirect uptake of CO_2-C fixed by plants, i.e. by photosynthesis products like root exudates and dead plant residues (Gavrichkova and Kuzyakov, 2017). Nevertheless, apart from plant assimilates, agricultural soils gain also C from manure addition (Ciais et al., 2010). For example, the plant biomass production (gross primary production, GPP) minus autotrophic respiratory losses (resulting in net primary production, NPP) of the arable land in 25 member states of the European Union[1] (EU-25) ranges between 490 and 846 g C m^{-2} yr^{-1}, depending on data sources (Ciais et al., 2010). When accounting for the C losses from heterotrophic respiration, harvest, erosion and burning of harvest residues (net biome production, NBP), the arable land of EU-25 loses between 8.3 and 13 g C m^{-2} yr^{-1}, with standard deviations of 13 and 33 g C m^{-2} yr^{-1}, respectively (Ciais et al., 2010). Thus, agricultural soils tend to act as a small source rather than as sink of CO_2 in Europe.

Generally, long-term cultivating results in significant decreases in SOC and SOM, respectively, compared to native soils (Schnitzer et al., 2006). At the same time, cultivation causes simultaneously an increased polymerisation which was thought to increase the molecular stability of SOM against enzymatic activities, e.g. C mineralisation (Schulten et al., 1995). However, studies that are more recent emphasise ecosystem properties, which control the microbial accessibility of SOM – sorptive protection by minerals and occlusion in aggregates – as the crucial factor for SOM turnover (Dungait et al., 2012; Lehmann and Kleber, 2015). Nevertheless, agricultural soils can potentially sequester C and mitigate CO_2 emissions, mainly by the adoption of appropriate tillage management, the use of crop rotations with high C input and organic amendments (Ogle et al., 2005). But for effective sequestering strategies, it has to be considered that these management improvements have to be maintained since the gains in soil C stock are reversible in case of a reversion to former management (Paustian et al., 2000). Additionally, soils have a finite capacity to accumulate C (Paustian et al., 2000) and the efficiency of sequestration in arable soils is much lower per unit of input than in other biomes due to tillage for instance (Ciais et al., 2010) (see section 1.4.1).

[1] EU with Great Britain and without Bulgaria, Croatia and Romania

With elevating atmospheric CO_2 concentration, the assimilation rate and water use efficiency of especially C_3 carbon fixation will probably increase and, thus, autotrophic SR. Simultaneously, heterotrophic SR is suggested to increase with C input from assimilates as well (Subke et al., 2006), resulting in a faster turnover of at least new C inputs (van Groenigen et al., 2017). On the downside, based on their model, Black et al. (2017) speculate that this increased input in combination with global warming will rather fuel the priming of old soil C than add new C to soils, while drought could counteract this effect by reduction of autotrophic SR. However, things are more complicated here; one has to consider that the combined effect of global change factors on SR may act additive, synergistic or antagonistic (Zhou et al., 2016). Hence, a lot of effort is needed to understand and to predict the overall impact of global change on SR or soil C cycles.

1.2.2 Nitrification, denitrification and nitrifier denitrification

In addition to its role as a potent GHG, N_2O became by now the dominant ozone-depleting substance (Crutzen and Ehhalt, 1977; Ravishankara et al., 2009). Besides nitrate (NO_3^-) leaching and NH_3 volatilization, N_2O emission is one of the most dominant N loss pathway from soils (Congreves et al., 2016). N_2O is produced in soils by microbial N turnover processes, mainly as by-product of nitrification or as intermediate of denitrification and nitrifier denitrification, which pathways may be interlinked, for example by coupled nitrification-denitrification (Wrage et al., 2001). Due to fertiliser application, agricultural soils are more prone to N_2O emissions than uncultivated soils. However, the latter are also affected by atmospheric N depositions from human activities (Fowler et al., 1998). In general, N_2O formation depends on the aeration status of a soil, which is controlled by O_2 supply and by respiratory O_2 consumption (Balaine et al., 2013; Parton et al., 1996). Soil water modulates aeration by hampering gas diffusion through soil pores. Hence for convenience, water-filled pore space (WFPS) is often used to characterise the aeration status of a soil, because it is relatively easy to obtain (Eqs. (1-2)–(1-4)) (Linn and Doran, 1984):

$$WFPS = \frac{\theta_v}{\varphi}, \tag{1-2}$$

where θ_v is volumetric water content ($cm^3\ cm^{-3}$) and φ is total porosity ($cm^3\ cm^{-3}$) with

$$\boldsymbol{\theta_v = \theta_g \times \rho_b}\ \textit{and} \tag{1-3}$$

$$\boldsymbol{\varphi = 1 - \frac{\rho_b}{\rho_p}}, \quad (1\text{-}4)$$

where θ_g is gravimetric water content (g g^{-1}), ρ_b is bulk density (g cm^{-3}) and ρ_p is particle density, which is assumed to be 2.65 g cm^{-3} (density of quartz).

Nitrification denotes the microbial oxidation pathway of NH_3 through the intermediates hydroxylamine (NH_2OH) and nitrite (NO_2^-) to NO_3^-. It was hypothesised to be a microbial reaction for the first time in the second half of the nineteenth century (Schlösing and Muntz, 1877). Autotrophic nitrifiers use nitrification to gain energy for CO_2 fixation, whereas heterotrophic nitrifiers use organic C as energy and C source (Wrage et al., 2001). During nitrification, N_2O originates from the incomplete oxidation of NH_2OH to NO_2^- by NH_2OH oxidoreductase (Arp and Stein, 2003). Further, the leakage of NH_2OH and its subsequent autoxidation and reaction with the soil matrix or its comproportionation with NO_2^- can produce N_2O abiotically (Heil et al., 2016; Cantera and Stein, 2007). Nitrification has always been considered to be performed in two steps by NH_4^+- and NO_2^--oxidising microorganisms. Only recently, the complete oxidation of NH_4^+ to NO_3^- within one organism (comammox) was identified in the bacterial genus *Nitrospira* (Figure 1-3) (Daims et al., 2015; van Kessel et al., 2015). Nitrification is reliant upon O_2 as terminal electron acceptor and its availability is controlled by relative soil gas diffusivity as a function of soil texture and water content (Balaine et al., 2013). For example, Bateman and Baggs (2005) and Davidson (1992) reported a WFPS between 35 and 60 % and a water content below field capacity, respectively, as favourable for nitrification. Further controls over N_2O production during nitrification are N turnover rate, NH_4^+ content and temperature of the soil (Parton et al., 1996; Farquharson, 2016). An increase in pH promotes the free NH_3 concentration (acid dissociation constant of NH_3 / NH_4^+ is 9.25), but pH values > 9.0 inhibit NO_2^- oxidation. Thus, a slight alkaline pH appears as the optimum for nitrification activity (Norton, 2008). Accordingly, nitrification is a significant process in acidic forest soils where fungi predominate (Barraclough and Puri, 1995). However, the contribution of nitrification to N_2O emissions is relatively small at rates of regularly less than 0.2 % of net nitrified N (Farquharson, 2016).

For denitrification, a water content above 70 % WFPS and near field capacity, respectively, is required (Davidson, 1992; Bateman and Baggs, 2005). However, hotspots of respiration caused by decaying organic matter can also create anaerobic microsites (Parkin, 1987). During denitrification, NO_3^- with an oxidation state for N of +V is reduced

sequentially to NO_2^-, nitric oxide (NO), N_2O and, finally, to gaseous nitrogen (N_2) with an oxidation state of zero (Figure 1-3) (Zumft, 1997). Denitrifiers use these N oxides as terminal electron acceptors under anaerobic and temporarily aerobic conditions to gain energy from microbial available organic C (Bouwman, 1996). This reduction of NO_3^- by bacteria was first described at the end of the nineteenth century by Gayon and Dupetit (1886). While N_2O is a potent greenhouse gas (see section 1.1), the generation of N_2 is innocuous from an environmental stance (Davidson et al., 2015). The stimulation and progression of the denitrification sequence and, thus, the relative share of denitrification-derived N_2O and N_2 in gaseous N losses from soil to atmosphere depend mainly on three proximate factors: availability of O_2, NO_3^- and C (Tiedje, 1988; Knowles, 1982). First, increasing O_2 concentrations inhibit the enzyme activity of the denitrification sequence in the order N_2O reductases (*nosZ*) < nitric oxide (NO) reductases (*qnorB* and *norB*) < NO_2^- reductases (*nirK* and *nirS*) < NO_3^- reductases (*narG* and *napA*). In other words, the lower the O_2 concentration, the further the sequence is processed by different forms of these relatively complex reductases (Schulthess et al., 1994; Tiedje, 1988; Philippot, 2002). The activity of these enzymes correlate with the production dynamics of N_2O and N_2 with decreasing redox potential: whereas a threshold below +300 mV is critical for denitrification, a maximal evolution of N_2O occurs at 0 mV, while the N_2O / (N_2O+N_2) product ratio decreases with a further decrease in redox value (Kralova et al., 1992; Masscheleyn et al., 1993). As NO_3^- gets reduced, the redox potential of a soil also decreases (Brettar et al., 2002), indicating the availability of NO_3^- as the second proximate regulator of denitrification (Tiedje, 1988; Knowles, 1982). A relative shortage increases the reduction of the successors as N_2O in the denitrification sequence, whereas the availability of NO_3^- and NO_2^-, respectively, increases the N_2O / (N_2O+N_2) product ratio (Firestone et al., 1979). This relationship is ascribed to the Michaelis-Menten kinetics during the respective reduction steps in the sequence. The reaction rate of reduction is higher for NO_3^- and NO_2^- than for N_2O, which results in an accumulation of N_2O if NO_3^- concentration is not limited by diffusion or nitrification rate for instance (Betlach and Tiedje, 1981). Further, the reduction of NO_3^- gains a higher energy yield than the reduction of NO_2^- and the latter in turn more than N_2O (Koike and Hattori, 1975), favouring also the reduction of available NO_3^- . The third control of denitrification activity is the availability of carbon as electron donor relative to the electron acceptor(s) (Tiedje, 1988; Knowles, 1982). Third, a high C availability relative to NO_3^- availability results in an electron surplus, which is used by

denitrifiers to reduce more N_2O to N_2 compared to an environment with a low C / NO_3^- ratio (Benckiser et al., 2015; Pan et al., 2013). In arable soils, most of the denitrification activity is attributed to bacteria. Although the contribution of fungal denitrification is smaller, its proportion increases with decreasing soil pH, since bacterial denitrification is inhibited at low pH (Herold et al., 2012). Nevertheless, fungal denitrification is of ecological significance because many fungi lack *nosZ*, resulting in N_2O as the dominant gaseous product (Laughlin and Stevens, 2002). A summary model by Hofstra and Bouwman (2005) based on 336 published measurements identified higher denitrification rates under grassland compared to cropland, which may be related to higher biological activity in grassland soils, caused by a higher availability of organic material. Likewise, poorly drained soils and increasing N fertilisation rates significantly foster denitrification (Hofstra and Bouwman, 2005).

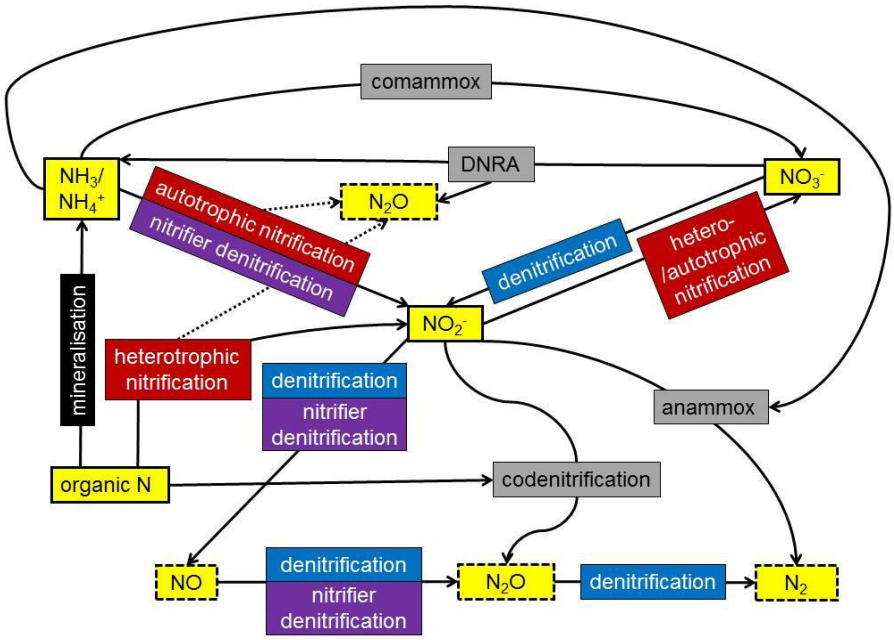

Figure 1-3. Processes and pathways of N conversions associated with N_2O and N_2 emissions in soils. Modified adaption from Wrage-Mönnig (in Kammann et al., 2017).

Nitrifier denitrification is a facultative pathway of nitrifiers in which NO_2^- is reduced to N_2 by NO and N_2O by the same autotrophic microorganisms and is the main N_2O source when moisture conditions are suboptimal for denitrification (Figure 1-3) (Wrage et al., 2001; Kool et al., 2011). Additionally, a high NH_4^+ supply at elevated NO_2^- concentrations as well as low carbon availability and low pH seem to promote this pathway (Wrage et al., 2001; Zhu et al., 2013; Kool et al., 2010). However, when the NO_2^- concentration is low, a high NH_4^+ abundance fosters rather nitrification (Wunderlin et al., 2012). Beside

its possible function to out-compete nitrite oxidisers for O_2 or as detoxification mechanism for excess nitrite, nitrifier denitrification has been suggested as an electron sink from the cytochrome pool to promote NH_2OH oxidation to NO_2^- and to prevent formation of nitrosating intermediates (e.g. NO) from NH_2OH autoxidation (Cantera and Stein, 2007). In agreement, recent studies suggest that processes like nitrifier denitrification are more important compared to nitrification and denitrification than previously assumed for gaseous N production from soils (van Groenigen, J. W. et al., 2015).

Other biotic processes that produce N_2O and N_2 in soils are codenitrification (mainly N_2O), ammonification of NO_3^- and NO_2^- (DNRA, only N_2O) and anaerobic oxidation of NH_4^+ with NO_2^- to N_2 (anammox) (Figure 1-3) (Spott et al., 2011; Rütting et al., 2011; Long et al., 2013). All of these processes seem to be fostered by anaerobic or at least hypo-aerobic conditions (Butterbach-Bahl et al., 2013). Codenitrification via N-nitrosation is mediated by a microbial reaction, which combines one N atom of NO_2^- or NO with one N atom of another N species, e.g. amino compounds (Spott et al., 2011). DNRA is highest in carbon rich and electron-acceptor-poor environments since it enables the accommodation of eight electrons during NO_3^- reduction to NH_4^+ in anaerobic environments (Tiedje, 1988). The N_2 produced by Anammox is formed from equimolar amounts of NH_4^+ and NO_3^- whereby NO_2^- is suggested as the direct oxidation agent of NH_4^+ (van de Graaf et al., 1995). Only recently, non-denitrifying *nosZII*-type bacteria capable of N_2O consumption in soil have been discovered (Domeignoz-Horta et al., 2016). Beside NH_2OH oxidation (see above), reduction of NO_3^-, NO_2^- and NO with iron(II) (chemodenitrification) at high pH and low O_2 as well as anaerobic oxidation of NH_4^+ with Fe(III) (Feammox) in highly weathered soils are possible abiotic processes releasing N gases (Zhu-Barker et al., 2015; Yang et al., 2012).

The spatial inhomogeneity of soils at nano-, micro-, meso- and macro-scales creates concentration gradients by a high spatial and temporal variability. In combination with changing environmental conditions, e.g. rapid sequences of local O_2 and NO_3^- depletion, hotspots and hotmoments with high microbial activity might appear (Kuzyakov and Blagodatskaya, 2015; van Groenigen, J. W. et al., 2015; Knowles, 1982). Hence biological sources of N_2O emissions might change quickly (Chen et al., 2016). Additionally, plants can modulate these spatial soil conditions with their roots by removing NO_3^- and water or by addition of carbon and the reduction of rhizosphere O_2 concentration by respiration (Tiedje, 1988), but can also emit N_2O themselves (Smart and Bloom, 2001).

Climate change probably slightly stimulate soil N cycle (Latt, 2016) and, therefore, enhance denitrification especially in agricultural soils since these systems are principally more charged with high N loads from fertilizer use (Orwin et al., 2015). Additionally to altered rainfall patterns, a higher concentration of atmospheric CO_2 will improve the water use efficiency of photosynthesis, which may lead to increased denitrification rates by higher soil moisture (Fowler et al., 2015). Additionally, elevated atmospherically CO_2 increases labile C input from plants and, thus, enhances microbial respiratory activity, both favouring denitrification (van Groenigen et al., 2011). However, with warming only, N_2O emissions are suggested to decrease due to lower soil water contents (Liu et al., 2016). Hence, ongoing research is also mandatory to understand and to predict the overall impact of global change on soil N cycles.

1.3 Effects of anaerobic biogas digestates on soil nutrient cycling and greenhouse gas emissions from soils

1.3.1 Characteristics of anaerobic biogas digestates

Anaerobic digestion (AD) is the turnover of organic substrates to biogas [about 70% methane (CH_4) and 30% CO_2] by a microbial consortium of four functional groups which act successively during the fermentation process: hydrolytic bacteria, acidogenic bacteria, acetogenic bacteria and methanogenic archaea (Insam et al., 2015). To maximize biogas production, easily degradable biomasses such as animal slurries, energy crops, the organic fraction of municipal solid waste and agro-industrial residues are used alone or in mixture (Tambone et al., 2010). Along with biogas, biogas digestate (BD) represents the final products of AD. Synonyms for BD used in the literature are ‘biogas (fermentation) residue’ or ‘biogas slurry’ if animal slurry is used as a substrate. The CH_4-rich biogas is used to produce electricity and heat, whereas the digestate could be utilised as amendment in agriculture because of its high nutrient content [N, phosphorus (P), potassium (K)] in available form and, thus, very good fertilising properties (Tambone et al., 2010). During AD, organic matter mineralises, which reduces the total and organic C content by 20–95 % due to microbial conversion into CH_4 and C, while complex organic N compounds like proteins are mineralised to NH_4^+ (Möller and Müller, 2012; Möller, 2015). These processes lead to a decrease in the C / N and an increase in the NH_4^+ to total N ratio, respectively. Together with a simultaneously upward shift of pH by 0.5–2.0 units during AD, the potential for NH_3 losses from BD increase (Möller and Müller, 2012). At the same

time, inorganic nutrients as P, K, magnesium (Mg) and calcium (Ca) remain preserved, resulting in an increase in concentration of the latter (Möller and Müller, 2012). Lignin-like material, complex lipids and steroids, which are potential humus precursors, also remain and concentrate during AD and hence have an important influence on SOM turnover (Lorenz et al., 2007). However, BD is a relatively new type of soil amendment, so its long-term stability in soil is still under debate, as recently reviewed by Möller (2015). Thus, the impact of BDs on soil properties has still to be monitored and evaluated in both short-term (initial stages) and long-term studies at the laboratory as well as the field scale (Insam et al., 2015).

1.3.2 Soil organic matter turnover in soils treated with biogas digestate

The organic fraction of BD is much more recalcitrant than the input feedstock due to the mineralisation processes during AD, although not the total easily degradable organic matter is degraded (Askri et al., 2015; Alburquerque et al., 2012a; Coban et al., 2015). More precisely, lipids, amides and polysaccharides are consumed, whereas thermostable compounds are enriched, accompanied by an increase in the degree of aromaticity (e.g. lignin compounds) and an accumulation of long-chain aliphatic components (Möller, 2015). As a consequence of the lower content of easy degradable C, slower growing microorganisms (*K*-strategists) will establish in the soil (Chen et al., 2012). Thus, continuous BD application reduces microbial growth rate and activity, resulting in a slower SOM turnover (Bachmann et al., 2014; Eichler-Löbermann et al., 2014; Johansen et al., 2013). However, since the microbial available C fractions, which are converted into biogas during AD, are principally correlated to the fractions of undigested organic matter, which are preferably decomposed by soil microorganisms, a similar SOM reproduction is anticipated – whether the amendment is digested or not (Möller, 2015; Insam et al., 2015; Thomsen et al., 2013). Contrary, AD could even increase the C retained in soil, at least for the initial 1–2 years (Thomsen et al., 2013). Nevertheless, in the short-term, a priming effect after application of BD has been observed, albeit it lasted shorter than the priming effect after the application of undigested slurry (Coban et al., 2015; Stumpe et al., 2012; Alburquerque et al., 2012a). Such a priming effect could be attributed to the relatively low C / NH_4^+ ratio of BDs if the biodegradability is relatively high, i.e. when the dissolved organic C to total N ratio is lower than 1.5 (Alburquerque et al., 2012a). However, Chen et al. (2011a) linked the bulk of this relatively high C losses to the evolution of CO_2 from dissolved inorganic C in the BD. Despite its high plant availability – in the short-term –

the increased NH_4^+ content of BD is also prone to losses by nitrification and microbial immobilisation (Alburquerque et al., 2012a; Alburquerque et al., 2012b). At the same time, these authors observed no effect of BD on soil N compared to mineral fertiliser or undigested cattle manure after two seasons of horticulture cropping (Alburquerque et al., 2012b). Moreover, one of the scarce 'long-term' studies, reports no effect on either total N or SOC stocks after 15 to 25 years of application of BD at five organic farming sites in Germany (Wentzel et al., 2015). Thus, up to now, there is no tangible evidence for an enhanced SOM degradation upon fertilisation with BD. Moreover, the build-up of SOM is generally a slow process over decades (Jenkinson and Rayner, 1977; Balesdent et al., 1988). Hence, the available data reflect on their own terms rather short studies and, consequently, more knowledge about the effect of BD on SOM stocks is necessary.

1.3.3 Carbon dioxide and gaseous N emissions from soils treated with biogas digestate

BDs may induce a lower SR due to their lower content of microbial available C compared to undigested organic amendments. Indeed, short-term studies confirmed that the heterotrophic SR is lower if BD is used (Stumpe et al., 2012; Johansen et al., 2013; Chen et al., 2012). Nevertheless, the magnitude of this effect may depend on the digested feedstock and the soil type (Abubaker et al., 2013a; Alburquerque et al., 2012a). After eight years of BD application, Odlare et al. (2014) found also a slightly but significantly lower basal respiration compared to the treatments with compost, manure or mineral fertiliser. Bachmann et al. (2014) observed lower enzyme activities in a digestate-amended soil compared to a soil with undigested amendment in a field study after three years. From that, they deduced a slower C turnover but found also no differences in soil CO_2 efflux between these two treatments. Nevertheless, these authors acknowledge that crop growth might have masked differences of heterotrophic microbial respiration by a high temporal variability of autotrophic root respiration. Accordingly, Hupfauf et al. (2016) found recently that plants affect the activity of soil microbes after BD application also due to nutrient competition. The dynamic of the CO_2 evolution from soils after BD application implies that the bulk of the easy degradable C is respired during the first few days. For example, in an incubation experiment by Coban et al. (2015), up to 40 % of total BD-C were respired within 7 days, whereas 35 % of this C was still recoverable after 378 days. In a short-term field study by Terhoeven-Urselmans et al. (2009), still 27 % of the BD derived C were recovered as CO_2 during a period of 50 days. Compared to the initial emission

peak after the application of undigested amendments, the cumulated CO_2 peak of BD is smaller because this peak lasts shorter (Stumpe et al., 2012; Chen et al., 2011a). However, the immediate CO_2 release from BD might be even higher (Thomsen et al., 2013), which could be attributed to the release of residual CO_2 from the AD process. Accordingly, Chen et al. (2011a) found dissoluted inorganic C from BD as an important source of CO_2 emissions directly after application. Overall, it seems reasonable to account the difference of the CO_2 emissions between soils with digested and soils with undigested amendments to the already generated CO_2 during AD (cf. section 1.3.2).

N_2O emissions from agricultural soils are directly linked to the addition of fertiliser N which promotes nitrification and denitrification (Mosier et al., 1998a). Emissions peaks of N_2O usually occur during the first week after fertiliser application, due to freeze-thaw cycles and after tillage (Dobbie et al., 1999; Drewer et al., 2017; Kaiser and Heinemeyer, 1996). With its increased NH_4^+ content and pH value, BD is supposed to enhance N_2O and NH_3 emissions from soils after application (Nkoa, 2014). However, available studies mostly suggest that at least N_2O emissions after the application of BD are rather smaller or at least do not change compared to the application of undigested amendments as reviewed by Insam et al. (2015) and Möller (2015). Presumably, the relatively low NO_3^- to C ratio in BD stimulates denitrification (Senbayram et al., 2012). However, this relationship depends also on the total N content of a BD and, further, on soil properties including soil water content, soil type and SOM content, which control the biological and chemical O_2 demand, resulting in increased N_2O emissions with lowered O_2 availability (Abubaker et al., 2013b; Clemens and Huschka, 2001). Alternatively, also the hampering of NH_3 oxidisers by specific phenols resulting from AD of especially pig manure are discussed as a reason for the observed reductions in N_2O emissions after BD application (Chantigny et al., 2007; Levén et al., 2006; Nyberg et al., 2004).

Although N_2O emissions seem to be lower rather than higher under specific soil conditions due to AD, the increased pH and NH_4^+ content of BD, however, evidently promotes NH_3 emissions after BD application, at least during the first hours after application when BD is not incorporated into soil immediately (Möller and Stinner, 2009; Quakernack et al., 2012). Emissions of NH_3 are of environmental concern due to acid deposition or conversion to N_2O for instance (Ferm, 1998; Mosier et al., 1998a). To reduce immediate NH_3 losses, the application of BD by injection is recommended as best practice. Nevertheless, its benefit on NH_3 saving could be counterbalanced by a simultaneously increased potential for N_2O emissions due to due a reduction of local O_2 availability in the injection slit

(Webb et al., 2010; Wulf et al., 2002). In accordance, Severin et al. (2015) found no differences between digested and undigested amendments in three different soils, but they observed that injection and the soil type had strong effects on N_2O emissions.
Recent studies, however, give indices that the lower N_2O emissions from BD amended soils are associated with an increase in N_2 emissions due to denitrification, at least during the first two to three weeks after BD application (Köster et al., 2015). In opposite to N_2O, N_2 is not a GHG, but its emission represents an N loss from agricultural soil systems, which reduces soil fertility and plant nutrition. Thus, the effect of BDs on N_2O and N_2 emissions, both in the short and the long-term should be focussed on.

1.4 Objectives: short-term impacts of soil cultivation activities on nutrient cycling and greenhouse gas emissions from soils amended with biogas digestate

1.4.1 Tillage: carbon and carbon dioxide

As suggested in section 1.2.1, tillage affects SR and C sequestration potential. Tillage is a measure to supress weeds and to enhance the return of nutrients to soil (e.g. from crop residues) by the promotion of mineralisation processes. In general, tillage disrupts soil macroaggregates and disturbs, thus, the formation of microaggregates within the former. Since the microaggregates are the entities which can occlude and protect organic matter against biological degradation in the long-term, tilled soils have a reduced potential to sequester C (Six et al., 1999). Macroaggregates are also a physical protection of included particulate organic matter (POM) that is not yet stabilised as organic matter in microaggregates. Tillage disrupts this protection and makes the included POM by contrast available for microbial decomposition (Figure 1-4) (Six et al., 2000). Beside a short-lived (few hours) but intense CO_2 pulse caused by the physical degassing of trapped CO_2 from broken-up clods, the increased availability of POM for soil microbes – accompanied by an improved aeration – results in an enhanced mineralisation and hence increased SR for up to several weeks (Reicosky et al., 1997; Reicosky and Archer, 2007; Rochette and Angers, 1999). Due to this altered mineralisation rates, the accuracy of temperature-driven SR models (Lloyd and Taylor, 1994) may be impeded. Likewise, the immediate short-term impact of the enhanced microbial SOM turnover after tillage is still not well understood at the molecular level. Especially, it is unknown how the amendment with BD affects the dynamic of SOM turnover and the involved SR after tillage operations. Therefore, the disturbing effect of tillage on the quality of temperature-driven SR models is

evaluated in section 2 and the effect of tillage on the turnover of molecular organic soil compounds is investigated in section 3. Both studies have their focus on the effects of amendment with BD.

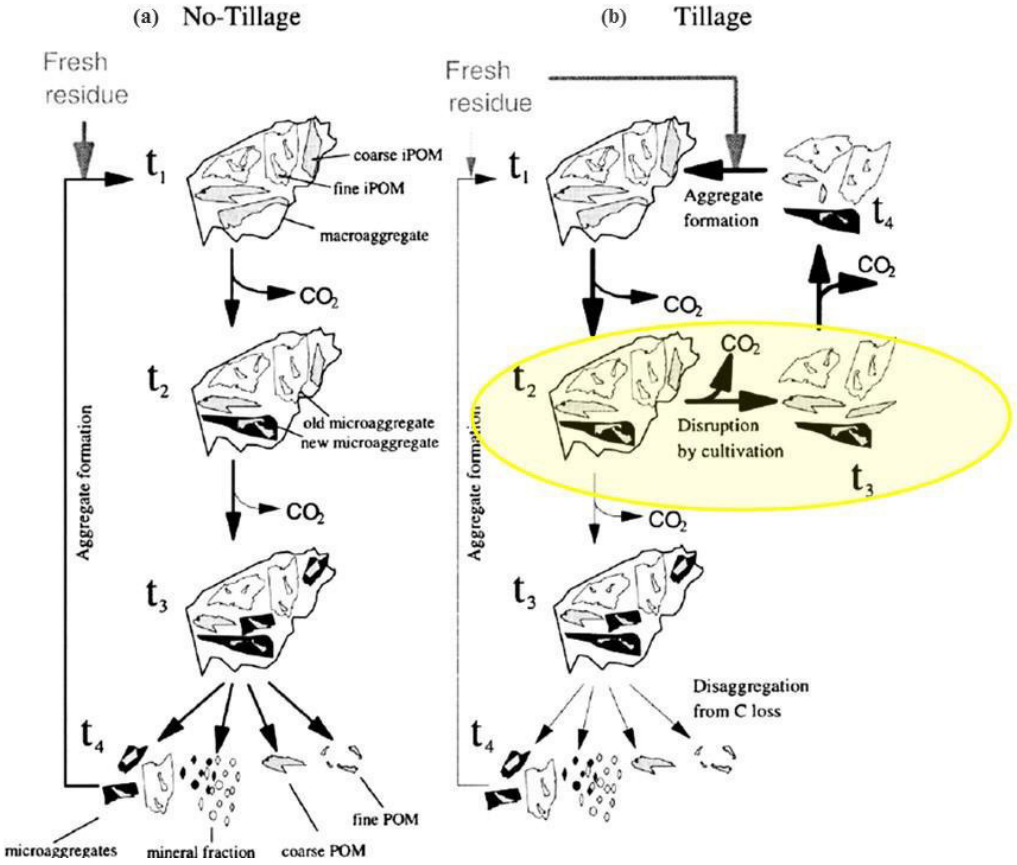

Figure 1-4. Conceptual model of macro- and microaggregate stability in a) not tilled and b) tilled soils. At t_1, fresh crop-derived residue is incorporated into macroaggregates and forms coarse intraaggregate particulate organic matter (iPOM). In not tilled soils, macroaggregates protect freshly encapsulated iPOM, which then decomposes to form new microaggregates inside the macroaggregate (t_1–t_3, left side). At t_4, C is depleted and microbial activity and production of binding agents decrease, leading to a potential disaggregation of the macroaggregates. In tilled soils, some macroaggregates go through the same sequence. However, a greater proportion of macroaggregates are disrupted by tillage in the field at t_2, resulting in a faster macroaggregate turnover rate. When iPOM is released from the aggregates, it becomes exposed to microbial decay, which leads to a loss of iPOM and an increased CO_2 flux in tilled compared to not tilled soils (t_2-t_3, right side). Note the less stable regime in tilled soils. Adapted from Six et al. (1999).

1.4.2 Application of fertiliser: nitrogen and nitrous oxide

High rates of gaseous N losses from agricultural soils are generally observed directly after fertiliser application and depend on soil characteristics like moisture and temperature (Dobbie et al., 1999). These losses from the soil system do not only reduce soil fertility and plant nutrition but can also cause adverse impacts on the environment, including acid rain from ammonia emissions and climate change by N_2O emissions (Cameron et al., 2013). However, different types of fertilisers induce different amounts of N_2O and N_2

losses. While mineral NH_4^+-containing fertilisers promote N_2O emissions from nitrification, organic fertilisers with their increased C contend might foster denitrification, which results in increased N_2 emissions (Dittert et al., 2005). As discussed earlier, the composition of BD is characterised by both high NH_4^+ and C contents, although the latter might be less easily available. This properties result in a commonly observed lag of N_2O emissions after BD application, which is most probably caused initially by the relatively low NO_3^- availability for denitrification and then – after a sufficient NO_3^- formation by nitrification – by the relatively low availability of C from BDs for denitrification (Petersen et al., 1996; Alburquerque et al., 2012a). Overall, direct emissions have been shown to be moderate for N_2O but high

for N_2 after BD application (Senbayram et al., 2012; Köster et al., 2015). However, these studies assumed a rather homogenously incorporation of BD into soil. In contrast, the injection of BD results in locally much higher nutrient concentrations and altered O_2 consumption and supply patterns, which might increase N_2O losses from denitrification (Wulf et al., 2002; Webb et al., 2010; Dell et al., 2011). Further, the interactive effects of high NH_4^+ contents from BD injection with soil structure, moisture and temperature on N_2 and N_2O emissions are unknown and have been evaluated in section 4.

2 Soil respiration after tillage under different fertiliser treatments – implications for modelling and balancing

Soil & Tillage Research 150 (2015) 30–42

Contents lists available at ScienceDirect

Soil & Tillage Research

journal homepage: www.elsevier.com/locate/still

Soil respiration after tillage under different fertiliser treatments – implications for modelling and balancing

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ARTICLE INFO

Article history:
Received 25 April 2014
Received in revised form 8 December 2014
Accepted 24 December 2014

Keywords:
Soil respiration
Soil organic carbon
Soil CO_2 flux modeling
Tillage
Hot-water extraction
Biogas residues

ABSTRACT

Temperature-driven models of soil respiration (SR) are crucial for estimating C-balances of arable soils. However, model construction may be severely influenced by tillage operations. The impact of tillage on the temperature dependence of SR was studied to reveal the temporal patterns of model quality of temperature-driven SR-models. To obtain SR, CO_2 fluxes were measured with a dynamic chamber technique in treatments of an energy crop rotation amended with biogas residues (BR) and mineral fertiliser (MF). Measurements were performed with short intervals during the first three days after tillage operations, then with extending intervals between measurements up to 35 days after tillage. Additionally, soil concentrations of hot-water extractable organic carbon (HWC) were determined before and during the experiment. Overall, in all treatments individual CO_2 fluxes were affected by the extent of soil disturbance and fertiliser treatment. The highest tillage-induced fluxes where observed after disking in MF treatment. Tillage also induced an immediate increase of HWC, indicating additional labile C and a fast response of microbial activity. However, the change of HWC lasted only one day and approximated the pre-tillage values within a week. Even though BR soil had a higher HWC content, the increased C mineralisation in one repetition of MF suggests that buried plant residues might have a higher influence on SR after tillage than the type of fertiliser. Directly after soil disturbance by tillage it was impossible to construct temperature-driven models for SR in all treatments. Assuming that the coefficient of determination is appropriate with $R^2 \geq 0.5$ and the model quality is good with NRMSE ≤ 0.15, the qualities of the models increased continuously with time, but were unsatisfying for at least two weeks. During this time, SR showed a high sensitivity to changing environmental influences like precipitation and soil moisture or available C for microbial turnover, rather than temperature. The treatment BR showed a less sensitive pattern, which might be attributed to an altered soil structure and microbial activity of soil after long-term application of an organic fertiliser like BR. Therefore, temperature-driven models for the prediction of soil derived CO_2 emissions should be applied carefully for the days and weeks after tillage and verification by measurements in shorter intervals is advisable.

1. Introduction

Soil respiration (SR) is an essential part of the terrestrial carbon cycle. As a large component of ecosystem respiration, SR is the main process of carbon transport from terrestrial ecosystems to the atmosphere (Bond-Lamberty and Thomson, 2010; Chen et al., 2011; Ryan and Law, 2005; Schlesinger and Andrews, 2000). In 2011, 37.8% of the global land area was used for agriculture (FAOSTAT, 2011). Thus, a large part of global SR is affected by agricultural land use (5.2 Mg C ha^{-1} yr^{-1}) (Chen et al., 2010; Raich and Schlesinger, 1992). In general, the composition and mass of three main C-pools control SR: (1) soil organic matter (SOM), (2) dead plant residues and (3) organic substances released by living roots (Kuzyakov, 2006). The contributions of these sources vary throughout the year (Atarashi-Andoh et al., 2012), depending mainly on soil temperature and moisture (Lloyd and Taylor, 1994; Raich and Schlesinger, 1992; Wang et al., 2000), affecting microbial activity (Álvaro-Fuentes et al., 2007; Borken et al., 2003; Kim et al., 2012).

In the long term, SR equals the total CO_2 efflux from biogenic sources in bare soils which is measured at the soil surface (Kuzyakov, 2006; Raich and Schlesinger, 1992). However, when short time intervals are considered, total CO_2 efflux may be much larger than SR,

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http://dx.doi.org/10.1016/j.still.2014.12.015
0167-1987/

e.g., due to short-term physical CO_2 release induced by management activities like tillage (Calderon and Jackson, 2002; Jackson et al., 2003; Rochette and Angers, 1999). Here, we will use the terms "CO_2 efflux" and "SR" to distinguish between the tillage-induced CO_2 emission and the permanent CO_2 emission that is largely constituted by SR. The CO_2 efflux of agricultural soils is influenced by various management factors, such as type and amount of fertilizer (Rochette and Gregorich, 1998; Sänger et al., 2010), cropping system (Ceschia et al., 2010; Osborne et al., 2010), tillage system (Ciais et al., 2010; Lopez-Garrido et al., 2009; Reicosky, 1997) and individual tillage operations (Osborne et al., 2010; Reicosky and Archer, 2007). Agricultural tillage operations enhance to a large extent CO_2 emissions from soils (Reicosky et al., 1997; Reicosky and Archer, 2007). Immediately after tillage, there is a sharp increase in soil CO_2 efflux due to the physical release of CO_2, followed by a rapid decrease after a few hours (Álvaro-Fuentes et al., 2007; Calderon and Jackson, 2002; Morell et al., 2010; Reicosky et al., 1997). This instantaneous CO_2 release is caused by gas accumulations in soil clods and aggregates, which are broken up by the tillage operation. After this first degassing, the increase of oxygen availability in the tilled soil promotes microbial activity, resulting in enhanced SR. In contrast to the first instantaneous degassing, the CO_2 efflux resulting from this aerobic microbial activity declines much slower with time (Ellert and Janzen, 1999; Jackson et al., 2003; Reicosky and Archer, 2007). In general, in temperate climate zones the tillage-induced CO_2 release from soil ends after a few days (Ellert and Janzen, 1999; Rochette and Angers, 1999).

Since the decay of SOM and organic residues is the only source of SR on bare soils (Kuzyakov, 2006), the temporal changes of SOC fractions are relevant when considering SR after tillage (Dungait et al., 2012). As carbohydrates like simple sugars and starch are the most easily biodegradable SOC fractions (Dungait et al., 2012; Kalbitz and Kaiser, 2008; Yano et al., 1998), it is supposed that tillage has an impact on the relationship between availability of these fractions and respiration. An appropriate approach to quantify labile C, especially carbohydrates like pentoses, hexoses and polysaccharides, in soil is hot-water extraction (Ghani et al., 2003; Leinweber et al., 1995; Sparling et al., 1998).

Precise information about the impact of management activities on soil CO_2 efflux is needed to estimate carbon budgets and greenhouse gas inventories (Ceschia et al., 2010; Lehuger et al., 2011). This applies especially to agricultural land, due to its diversity in terms of crops grown, rotation, management, soil types, and climatic conditions (Osborne et al., 2010; Smith et al., 2010). Soil CO_2 efflux is commonly measured using chamber-based methods (Gesch et al., 2007; Pumpanen et al., 2004). Measured fluxes are then used to construct models that allow for the estimation of soil CO_2 efflux for the periods between measurement dates (Beetz et al., 2013; Elsgaard et al., 2012). A common approach for modelling soil CO_2 efflux is the use of an exponential function describing its relationship with soil temperature (Lloyd and Taylor, 1994). From a biogeochemical perspective this approach is only valid when the soil CO_2 efflux represents pure SR. The temperature model has to be calibrated against site-specific conditions. This is commonly done using continuous data of temperature and soil CO_2 efflux for the full range of one day, encompassing the lowest and highest soil and air temperatures during the day. However, this approach is feasible only for undisturbed site conditions (especially regarding temperature, soil moisture, and developmental stage of crops). Thus, directly after disturbance of the soil by tillage, a relationship between temperature and soil CO_2 efflux cannot be established using this approach and consequently, the temperature models for soil CO_2 efflux, which are based on data recorded on undisturbed soils, cannot be applied immediately after tillage operations. Thus, the altered CO_2 fluxes increase the uncertainty in the modelling of the overall CO_2 balance of crop rotations (Osborne et al., 2010; Zhang et al., 2011a). Until now, some basic approaches have been proposed to quantify the effect of tillage on soil CO_2 efflux. In such models a correlation of SOC decay and SR is assumed and both are modeled using a first order kinetic approach (Ellert and Janzen, 1999; La Scala et al., 2008, 2009; Teixeira et al., 2010). However, the parameters needed for such models are soil and site specific and are a priori unknown for a given site. This hampers the application of such approaches for in situ observations.

It is of great practical significance to know the duration of the "disturbed" state with CO_2 effluxes governed by the soil tillage operation in order to (1) estimate the amount of CO_2 that is released after the tillage operation, and (2) determine when the estimation of CO_2 efflux with a temperature-driven model can be resumed after tillage. The use of different fertiliser types with their different proportions of organic matter leads to different emissions from soil. The emissions arising from the use of novel fertilisers like biogas residues (BR) are of particular interest (Eugster et al., 2010; Sänger et al., 2010). This may also affect the emissions after tillage. We are not aware of any studies dealing (1) with tillage induced CO_2 emissions from arable fields fertilised with BR and (2) with the recalibration of temperature driven models of SR after soil disturbance. To overcome this shortage, we investigated the extent and temporal evolution of CO_2 emissions under different fertiliser regimes as affected by tillage operations. This is important in order to establish and to (re-) calibrate models of SR to obtain carbon balances of agricultural sites.

2. Material and methods

2.1. Study site

The study site is located in northeast Germany's terminal moraine of the Weichselian glacial period at 53°48′35″N and 12°4′20″E (elevation 10 m) within a gently rolling relief. The soil is a stagnic luvisol (IUSS Working Group WRB, 2006) with loamy sand texture overlying bedrock of till. According to our own measurements the top soil (0–30 cm) had an organic carbon content of 1.02% (measured with CN-analyser "vario MAX", Elemtar, Hanau, Germany), pH of 6.8 (measured in H_2O with pH meter "CX-401", Elmetron, Zabrze, Poland) and bulk density of 1.45 g cm^{-3} (measured on 250 cm^3 soil cores). The climate is characterized by maritime influence with an annual average temperature of 8.5° C and an annual total precipitation of 569.4 mm for the 30 year period from 1982 until 2011 (LFA 2012). The experiment was conducted on a field which had been cultivated with a rotation of energy crops for the past seven years. The crop rotation consisted of maize (*Zea mays* L.), rye (*Secale cereale* L.), sorghum (*Sorghum bicolor* (L.) Moench), winter triticale (× *Triticosecale* Wittm.), ryegrass (*Lolium perenne* L.) and winter wheat (*Triticum aestivum* L.) and was cultivated to produce feedstock for biogas production. The size of the experimental plots was 4.5 by 22.5 m.

Five days following the whole-plant harvest of winter wheat (27 July 2012), the field site was prepared for mustard as catch crop with the following management measures: liming (1 t CaO ha^{-1}) and deep loosening with deep loosener "Amazone TL" (40 cm depth) plus disking with disk harrow "Väderstad Carrier 300" (up to 10 cm depth) on the first day (1 Aug 2012). On the second day (2 Aug 2012), the preparation was continued with mouldboard ploughing and packing with reversible plough "Överum CX 490" plus packer (30 cm depth). Subsequently, drilling of mustard as catch crop with seedbed combination "Lemken System-Kompaktor S" (8 cm depth) concluded the tillage (Table 1). The mustard emerged on 8 Sep 2012.

Three fertiliser treatments were compared: (1) mineral fertiliser (MF), (2) biogas residue (BR) and (3) a mixture of 50%

Table 1
Depth, date ("day 1" = 1 Aug 2012) and time of tillage operations deep loosening (DL), disking (DS), mouldboard ploughing (MP) and drilling (DR) at the experimental site for the treatments with mineral fertiliser (MF), biogas residues (BR) and combined with 50% MF and 50% BR (MFBR).

		Date and time	
Tillage operation	Depth (cm)	Treatment MF	Treatments MFBR and BR
DL	40	Day 1, 11:30	Day 1, 10:45
DS	10	Day 1, 14:30	Day 1, 14:00
MP	30	Day 2, 9:15	Day 2, 8:30
DR	8	Day 2, 11:30	Day 2, 10:45

MF and 50% BR (MFBR). Whereas the BR and MFBR plots were located next to each other, the MF plot was at a distance of 50 m from the other two plots. In all treatments, equal overall amounts of N were applied (210 kg ha^{-1}). The feedstock for digestion in the biogas plant consisted of 91% cattle slurry, 7% rye groats and 2% maize silage. According to LFA (2012) we assumed, that 70% of the total N in the resulting BR was plant-available. The MF consisted of a mixture of ammonium sulphate nitrate (ASN) and calcium ammonium nitrate (CAN). The content of mineral N in the soil (0–60 cm depth) was 30 kg ha^{-1} before the first fertiliser application. The fertilisers were applied on 9 Mar 2012 (60% of BR and 44.4% of MF), 10 Apr 2012 (40% of BR and 33.3% of MF), and – for mineral fertiliser only – on 29 May 2012 (22.2%). On average, BR fertiliser featured a pH of 8.4, and original matters of C, NH_4–N, and total N of 3.35%, 0.3% and 0.48%, respectively. These differed slightly between the application dates due to different dry matter contents of 6.37 and 7.60% on 9 Mar and 10 Apr respectively.

2.2. Environmental parameters

During each measurement run we recorded soil temperatures in 2, 5 and 10 cm depth as well as air temperatures in 20 cm height and inside the chamber (°C) with portable sensors (AD592CN, Analog Devices, Norwood, MA, USA). Next to the field site, weather and soil data were recorded with an automatic meteorological station (DALOS 535, F&C, Gülzow, Germany) every 30 min, including air temperatures (°C) at 20 and 200 cm and precipitation (mm). Appertaining to the station, a pair of sensors for soil temperature in 2, 5 and 10 cm depth (AD592CN, Analog Devices, Norwood, MA, USA) and for volumetric (%) soil water content (SWC) (10HS, Decagon Devices, Pullman, WA, USA) in 10 cm depth were installed permanently in the MFBR treatment. During the tillage operations, these sensors were removed for six days. To obtain SWC by approximation for this gap, data obtained by soil samples (V = 250 cm^3) taken at 10 cm depth on days 2, 3, 9, 28 and 31 after initial tillage were interpolated linearly. The SWC sensor was calibrated with the SWC data of the 9th, 28th and 31st measurement date.

2.3. Measurement and estimation of CO_2 efflux

Measurements of CO_2 efflux started in the morning of 1 Aug 2012, at the first day of the tillage operations. The CO_2 flux measurements where performed with two LI-COR (Inc., Lincoln, NE, USA) LI-820 infrared gas analyzers, each connected to a dynamic square chamber with an area of 0.62 m^2 and a height of 0.55 m, resulting in a chamber volume of 0.341 m^3. For the measurements the chambers were placed on collars of 15 cm height, which were permanently installed into the soil to 12 cm depth. Three collars were placed 1 m apart from each other within each treatment.

The measurements started generally within 1 min after tillage operations by inserting collars and putting on the airtight opaque chambers immediately. Due to the successive measurement of the replicates in each treatment, we obtained pseudo-replications. The chambers were equipped with small fans (80 × 80 × 25 mm, 3000 rpm, 68 $m^3 h^{-1}$) in order to mix and homogenize the air inside the chambers. On the first two days, immediately after tillage operations, measurements were performed continuously for approximately half an hour. Afterwards the measurements were run approximately one to two times an hour. On the third day, we measured hourly in the morning and then with longer intervals between single measurements during the following days, i.e., two or three measurements on days 4, 5, 7, 8, 9, 28, 29 and 31. On days 13, 15, 27, 30 and 35 the CO_2 efflux was monitored on a sample basis, i.e., one measurement was taken. Finally, a whole-day campaign finished the experiment on day 36 (5 Sep 2012). During chamber placement, we recorded CO_2 concentrations in the chamber headspace with 1.3 s intervals for approximately 60 s, resulting in 46 data points per minute. Fluxes were estimated with function *fluxx* of package flux version 0.3–0 (Jurasinski et al., 2014) for the R statistical software version 2.15.2 (R Core Team, 2013). In short, the algorithm identifies the most linear part of the CO_2 concentration development during chamber placement time and fits a linear regression model (Eq. (1)):

$$f = \frac{MpV\mathrm{dc}}{RTA\,\mathrm{dt}}10^6 \quad (1)$$

with f the CO_2 flux (g $m^{-2} h^{-1}$), M the molar mass of CO_2 (44 g mol^{-1}), p the air pressure (Pa), V the chamber volume (m^3), R the gas constant (8.31 J $mol^{-1} K^{-1}$), T the temperature inside the chamber (K), A the area covered by the chamber (m^2), and dc/dt the CO_2 concentration change over time (ppm h^{-1}). The minimum proportion of data points to be kept for regression analyses was 50% of a concentration measurement to discard data noise at the beginning and the end resulting from chamber deployment and removal (for details see help file for function *fluxx* of package flux). Thus, each CO_2 flux was estimated at least from 23 concentration measurements. Only linear fluxes with a concentration change of at least 15 ppm, a normalised root mean square error (NRMSE) ≤ 0.2 and a coefficient of determination (R^2) of at least 0.75 were included in further analyses. We assumed linearity of concentration change and did not test for non-linearity since 96.3% of the obtained linear regressions had $R^2 \geq 0.95$.

2.4. Hot-water extraction of SOC

Composite soil samples (consisting of five individual subsamples, sharing distances of 25 cm) were taken from the upper soil layer (0–30 cm depth) with a gouge auger (3 cm in diameter) on the day before tillage (31 July 2012) and on the days 1, 2, 3, 4, 5, 7, 27, 31 and 35 after initial tillage (1 Aug–5 Sep 2012). After air-drying, the samples were sieved to 2 mm. For hot-water extraction, 25 g soil were boiled in 50 ml deionized water for 60 minutes (Leinweber et al., 1995). After filtration with pleated filter (240 mm, 80 g m^{-2}) by Munktell (Falun, Sweden), extracts were analysed with a DIMATOC 2000 (Dimatec, Essen, Germany) for determination of hot-water extractable organic C (HWC). This measurement of organic C is based on the principle of thermal-catalytic oxidation with subsequent NDIR detection. For each HWC sample, two replicates were analysed.

2.5. Modelling

Measured CO_2 flux data were fitted to an Arrhenius type equation (Lloyd and Taylor, 1994) (Eq. (2)).

$$\mathrm{SF} = R_{\mathrm{ref}} \exp\left[E_0\left(\frac{1}{T_{\mathrm{ref}} - T_0} - \frac{1}{T - T_0}\right)\right] \quad (2)$$

with SF the estimated soil efflux rate (mg CO_2–C $m^{-2}\,h^{-1}$), R_{ref} the respiration at the reference temperature (mg CO_2–C $m^{-2}\,h^{-1}$), E_0 an activation like parameter (K), T_{ref} the reference temperature (283.15 K), T_0 a notional temperature constant for the start of biological processes (227.13 K), and T the temperature in 2, 5 or 10 cm depth of soil or of air in 20 cm height or within the chamber (K). Which of those temperature data were used, depended on which yielded the best SF model for the data subset in question. When the data of one measuring day yielded no significant relationship between SF and T, we pooled the data of three consecutive measuring days and fitted the model again.

Statistical performance indicators were evaluated from regression analyses of observed and modeled data. This evaluation included the significance of models by *F*-tests, R^2 and normalized root mean square error (NRMSE). The coefficient of determination is assumed to be appropriate with $R^2 \geq 0.5$ and good with $R^2 \geq 0.75$ (Hoffmann et al., 2015). The NRMSE is a measure of the relative difference between modeled and observed values, normalized by the mean observed value (Eq. (3)), such that $0 < \mathrm{NRMSE} < 1$.

$$\mathrm{NRMSE} = \sqrt{\left[\sum_{n=1}^{n}\left(\frac{(M_i - O_i)^2}{n}\right)\right]}\frac{1}{\overline{M}}, \quad (3)$$

with n the number of observations, M and O the modeled and the observed values and $\overline{M}$ the mean of modeled values (Jorgensen et al., 1986; Mayer and Butler, 1993; Smith et al., 1996). The model is assumed to be in good agreement with observations when $\mathrm{NRMSE} \leq 0.15$, appropriate with $0.15 \leq \mathrm{NRMSE} \leq 0.3$ and poor with $\mathrm{NRMSE} \geq 0.3$ (Liu et al., 2013).

2.6. Statistical analyses

All statistics were run using the *R* statistical software version 2.15.2 (R Core Team, 2013). To evaluate the difference of CO_2 fluxes between the treatments by *F*-ratio, we performed a permutation test by Monte Carlo sampling with function *diffmean* of package simba (Jurasinski, 2012) for "R" since in some periods the number of measurements were rather small (Jurasinski & Kreyling, 2007). The original difference between the CO_2 fluxes of the compared treatments is compared against a distribution of 1000 differences that result from randomly re-assigning the measured fluxes to the treatments. We tested HWC for difference between treatments using the Mann–Whitney *U* test because of non-normal distribution. The CO_2 fluxes before and immediately after tillage were also tested for difference by Mann–Whitney *U* test because they were not normally distributed and because the number of repetitions were small ($n \approx 3$). We tested against a significance level $\alpha = 0.05$ in all tests.

3. Results

3.1. Weather conditions

During the study period, the average daily mean air temperature at 20 cm height was 17.4 °C, with a range between 13.2 °C and 25.6 °C. Overall precipitation was 35.9 mm, of which 17.6 mm were recorded during the first 8 days of the experiment (Fig. 1). The long-term average (1982–2011) values for this period of the year are 16.9 °C and 74.2 mm, respectively (LFA, 2012). Thus, during the study period in 2012 temperatures were on average slightly higher and precipitation appreciably lower compared to long-term records.

3.2. Carbon dioxide fluxes

Directly before liming and the following tillage operations, all treatments showed similar mean fluxes between 0.24 and 0.28 g $CO_2\,m^{-2}\,h^{-1}$ (Table 2). After liming, the fluxes of the treatment BR decreased slightly, whereas the fluxes of the MF treatment decreased markedly to 0.15 g $m^{-2}\,h^{-1}$. Immediately after the first tillage operation, i.e., deep loosening (DL), the fluxes in general increased to 0.27 (MF), 0.28 (BR) and 0.57 g $m^{-2}\,h^{-1}$ (MFBR). After disking (DS), the relative differences between treatments diminished while the mean fluxes increased to values between 0.65–0.79 g $m^{-2}\,h^{-1}$ (in the order BR < MF < MFBR). The single highest flux was detected in the MFBR treatment (1.01 g $m^2\,h^1$). After mouldboard ploughing (MP) and drilling (DR) (second day), CO_2 fluxes changed only slightly. Immediately after mouldboard ploughing, the fluxes decreased slightly in MF and MFBR (with a range 0.22–0.32 g $m^{-2}\,h^{-1}$ in the order MF < MFBR < BR). The change after DR was a modest increase again, which was quite pronounced for treatment MF, where fluxes up to 0.39 g $m^{-2}\,h^{-1}$ where detected. The ratio of post- to pre-tillage CO_2 fluxes ranged from nearly 1 to a little more than 3. The ratio was highest after disking in the treatment MFBR, and lowest after mouldboard ploughing in the treatment MF.

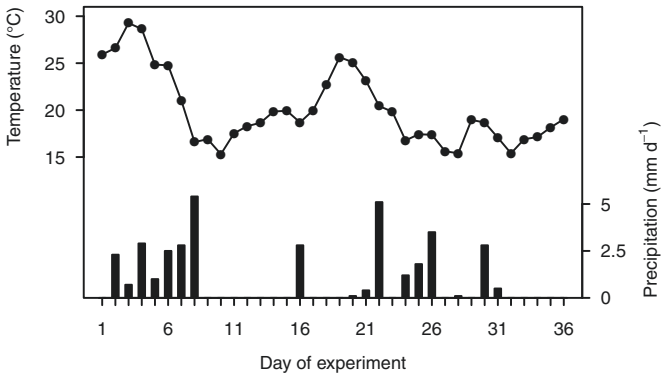

Fig. 1. Daily mean soil temperature in 5 cm depth (dots with lines) and daily precipitation (bars) during the studied period (1 Aug–5 Sep 2012).

Overall, CO_2 efflux of all three treatments showed large temporal variability, which seems to be mainly induced by management events and soil moisture behaviour related to precipitation events (Fig. 2). Measured fluxes in the MF treatment were somewhat different compared to the other two treatments over the days after tillage operations. In particular, fluxes in MF showed consistently higher standard deviations (SD) per day until day 27. This was especially evident on day 9, with fluxes ranging from 0.34 to 1.72 g $m^{-2}\,h^{-1}$. Simultaneously with this large range, the highest peak fluxes of the studied period were observed while the soil moisture had reached a maximum of 12.7% by volume. The fluxes from treatment MFBR were significantly higher than those from the others after tillage on the first day (permutation test, $p < 0.05$). When all tillage operations were finished, the order reversed and treatment MFBR showed the lowest fluxes which differed significantly from the others throughout the experiment (Table 3).

3.3. Hot-water extractable SOC

The values of HWC changed immediately and profoundly after tillage (Fig. 3). Before tillage, HWC contents were 0.028, 0.033 and 0.034% of soil dry matter for MF, MFBR and BR, respectively. On the first day, there was nearly a doubling of HWC in treatment MFBR and an even higher increase in BR. The treatment MF showed only a slight increase. On the second day, HWC decreased again below the values measured before the tillage operations in all treatments. Thereafter, HWC was similar to the initial values throughout the

Table 2
Mean CO_2 fluxes ($g\,CO_2\,m^{-2}\,h^{-1}$) before and immediately after liming, deep loosening (DL), discing (DS), mouldboard ploughing (MP) and drilling (DR). Values in brackets denote the standard deviation among the repetitions (n). Means with the same letter within the same column were not significantly different from each other (Mann–Whitney U test, $p < 0.05$). Liming was not tested due to insufficient number of valid repetitions.

Treatment	Before management	Directly after management				
		Liming	DL	DS	MP	DR
MF	0.25 (0.01) $n=3^a$	0.15 (0.01) $n=2$	0.27 (0.01) $n=6^a$	0.66 (0.1) $n=4^a$	0.22 (0.04) $n=4^a$	0.39 (0.05) $n=3^a$
MFBR	0.25 (0.04) $n=3^a$	0.26 $n=1$	0.57 (0.06) $n=4^b$	0.79 (0.16) $n=6^a$	0.25 (0.08) $n=3^a$	0.29 (0.07) $n=3^a$
BR	0.28 (0.01) $n=3^a$	0.23 (0.01) $n=2$	0.38 (0.06) $n=6^c$	0.65 (0.02) $n=6^a$	0.32 (0.02) $n=4^b$	0.38 (0.01) $n=2^a$

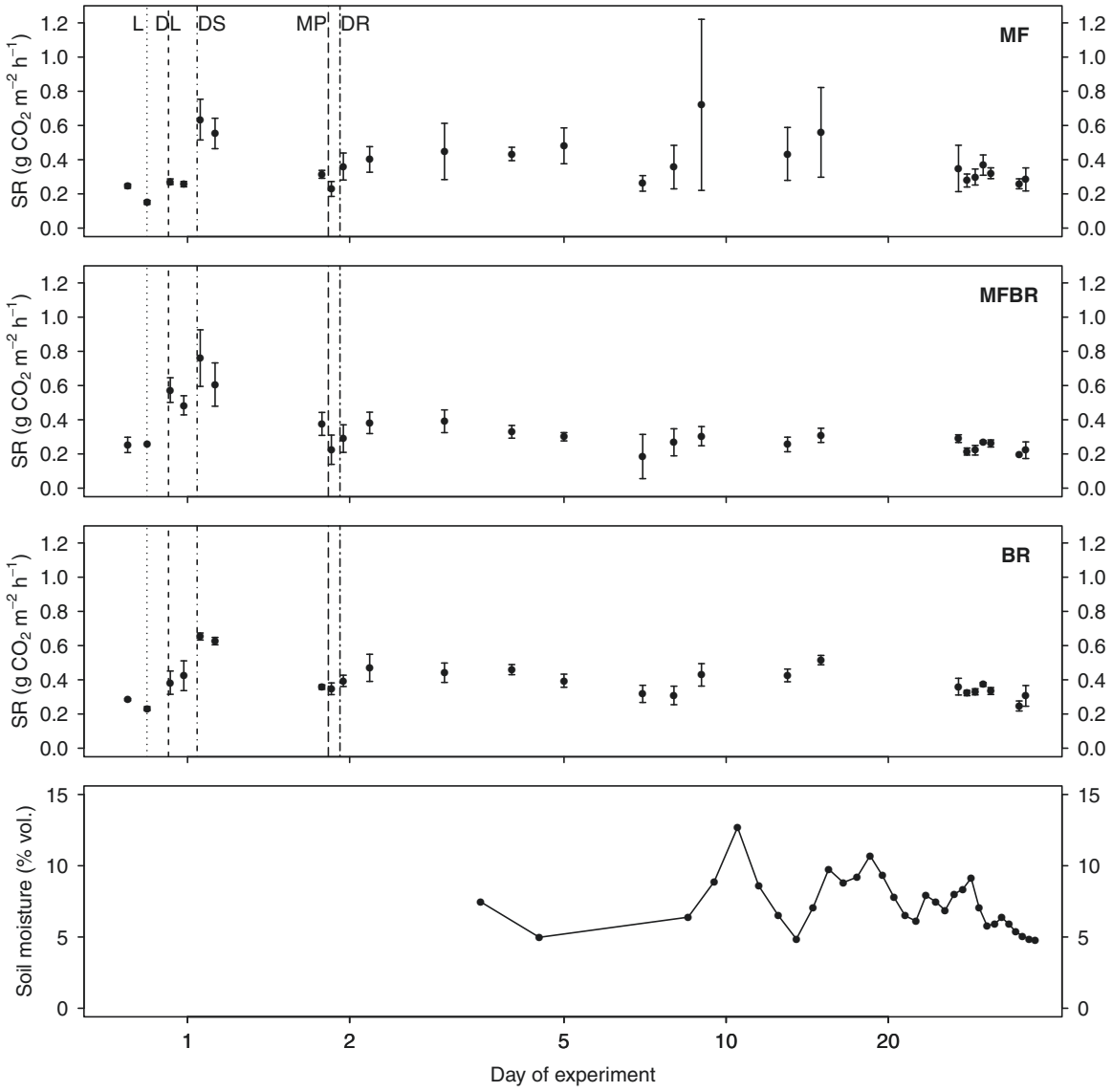

Fig. 2. Mean CO_2 fluxes before and after tillage. For CO_2 fluxes and soil moisture in 10 cm depth, daily mean values are shown. Fluxes before and immediately after tillage of the first two days are shown separately. Error bars show the standard error and vertical lines denote soil management and tillage operations: liming (L), deep loosening (DL), disking (DS), mouldboard ploughing (MP) and drilling (DR).

Table 3
Mean fluxes ($g\,CO_2\,m^{-2}\,h^{-1}$) and SD (in brackets) of the treatments for single days after tillage. Means with the same letter within the same column were not significantly different from each other (permutation test, $p < 0.05$).

Treatment/day	1	2	3	8	9	28	29	36
MF	0.59 (0.1)^a	0.31 (0.1)^a	0.45 (0.16)^a	0.36 (0.13)^a	0.72 (0.5)^a	0.28 (0.04)^a	0.30 (0.05)^a	0.28 (0.07)^a
MFBR	0.72 (0.16)^b	0.31 (0.1)^a	0.39 (0.07)^b	0.27 (0.08)^b	0.3 (0.06)^b	0.21 (0.02)^b	0.22 (0.03)^b	0.22 (0.07)^b
BR	0.64 (0.02)^c	0.4 (0.07)^b	0.44 (0.06)^a	0.31 (0.05)^{ab}	0.43 (0.07)^c	0.32 (0.02)^c	0.33 (0.02)^c	0.31 (0.06)^a

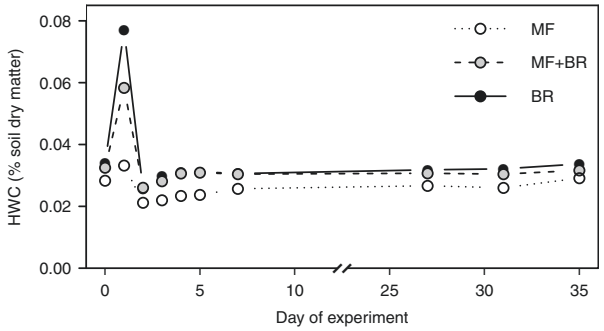

Fig. 3. Temporal development of HWC in soil in the treatments after tillage. On the first and the second day, the soil samples were taken approximately 3 h after the tillage operations resumed.

experimental period. The HWC values of treatment MF varied significantly from both MFBR and BR (Mann–Whitney *U* test, $p < 0.01$). However, the values of MFBR and BR did not vary significantly from each other ($p > 0.3$). In general, the HWC contents were decreasing in the order BR > MFBR > MF.

3.4. Model evaluation

The temperature driven model of soil CO_2 efflux described in Eq. (2) was applied to all treatments and showed temporal dynamics for each of them. Directly after tillage on the first day, no model fit with satisfactorily high R^2 was attainable in any treatment, neither with one of the recorded temperatures of soil nor with that of air. For the fluxes of the second and the third day, the models of all treatments showed only weak coefficients of determination ($R^2 < 0.5$) and were partly not significant (Table 4). The first period after tillage with a significant and acceptable model fit ($R^2 \geq 0.5$) was "day 15, 27, 28" in the treatments MF and MFBR. In the treatment BR, a good fit ($R^2 \geq 0.75$) associated with a high significance was already observed one pooling period earlier ("day 13, 15, 27"). In general, the qualities of model fit (increasing R^2) increased over the period after tillage. The treatment BR differed from the other treatments during the first week. Although there was already a high R^2 of 0.785 in the period "day 4, 5, 7", it decreased to 0.199 in the period "day 7, 8, 9" and increased again to the end of the study in accordance with the other two treatments. Considering the model quality, NRMSE values indicated increasing model quality with time in accordance with increasing R^2. However, after tillage, NRMSE was relatively high in treatments MF and MFBR, respectively. In the latter, model quality increased continuously and reached a good value (NRMSE < 0.15) in the period "day 13, 15, 27, 28". But NRMSE was relatively high (0.247–0.346) for most periods in the former. Here, NRMSE values decreased only on the last day of measurements to a good model quality. Compared to the other treatments, the values of NRMSE differed little during the experiment in treatment BR. In general, treatment BR had the lowest NRMSE of all treatments during most of the periods and showed acceptable or good model qualities from the second day throughout the experiment (Fig. 4).

Until "day 5, 7, 8", best model fits were found with soil temperature at 10 cm depth as the independent variable. For later periods, best model fits were found for soil temperatures at 2 cm depth as the independent variable, except for treatment MF, where

Table 4
Summary of the models (Eq. (2)) for the different treatments and periods: selected temperatures of the different soil depths (cm) or air in 20 cm height which provided the best model fit (T_{best}), coefficient of determination (R^2), significance values (p), normalised root mean square error (NRMSE), parameters (R_{ref} and E_0), temperature coefficient (Q_{10}), range of measured temperatures (T_{min} and T_{max}) and number of flux data for the model calculation (n).

MF	Period	T_{best}	R^2	p[b]	NRMSE	R_{ref} (g CO_2 h^{-1} m^{-2})	E_0 (K)	T_{min} (°C)	T_{max} (°C)	n
	Day 1	No fit						25.8[c]	28.7[c]	13[d]
	Day 2	$Soil_{10}$	0.319	**	0.247	0.077	522.7	16.9	22	21
	Day 3	$Soil_5$	0.127		0.329	0.256	173.6	19.3	27.9	13
	Day 4, 5, 7	$Soil_{10}$	0.31	*	0.256	0.142	442.4	16.1	20.2	15
	Day 5, 7, 8[a]	$Soil_{10}$	0.469	**	0.346	0.042	1069	15.4	18.2	19
	Day 7, 8, 9	No fit						15.7[c]	20.0[c]	29
	Day 13, 15, 27	Air	0.279		0.341	0.051	643	21.5	25.3	9
	Day 15, 27, 28	$Soil_{10}$	0.497	**	0.307	0.136	373.7	15.6	25	18
	Day 36	$Soil_2$	0.637	***	0.138	0.169	193.2	14.1	26.1	15
MFBR	Period	T_{best}	R^2	p[b]	NRMSE	R_{ref} (g CO_2 h^{-1} m^{-2})	E_0 (K)	T_{min} (°C)	T_{max} (°C)	n
	Day 1	No fit						24.8[c]	28.5[c]	14[d]
	Day 2	$Soil_{10}$	0.107		0.303	0.114	349.5	17.7	22.3	18
	Day 3	$Soil_{10}$	0.351	*	0.132	0.215	226.7	18.3	23.8	14
	Day 4, 5, 7	$Soil_{10}$	0.459	*	0.274	0.059	652.1	16.1	20.4	13
	Day 5, 7, 8	$Soil_5$	0.459	**	0.272	0.029	1000.8	15.7	18.9	17
	Day 7, 8, 9	$Soil_2$	0.378	**	0.26	0.083	467	16	22.5	25
	Day 13, 15, 27	Air	0.093		0.125	0.163	162.7	22	24.9	9
	Day 15, 27, 28	$Soil_2$	0.549	***	0.322	0.14	236.8	17.8	30.2	18
	Day 36	$Soil_2$	0.731	***	0.109	0.122	211.7	15.5	25.3	11
BR	Period	T_{best}	R^2	p[b]	NRMSE	R_{ref} (g CO_2 h^{-1} m^{-2})	E_0 (K)	T_{min} (°C)	T_{max} (°C)	n
	Day 1	No fit						24.9[c]	25.9[c]	14[d]
	Day 2	$Soil_{10}$	0.447	**	0.132	0.145	362.6	17.3	22.3	20
	Day 3	$Soil_{10}$	0.292	*	0.104	0.292	157.3	18.3	23.8	14
	Day 4, 5, 7	$Soil_{10}$	0.785	***	0.083	0.143	426.9	16.2	20.5	13
	Day 5, 7, 8[a]	$Soil_{10}$	0.361	*	0.161	0.157	379.3	15.7	18.3	18
	Day 7, 8, 9[a]	$Soil_5$	0.199	*	0.213	0.205	273.2	15.7	20.4	25
	Day 13, 15, 27	$Soil_2$	0.806	**	0.072	0.127	300.2	23.4	29.8	9
	Day 15, 27, 28	$Soil_2$	0.775	***	0.219	0.133	339.7	17.6	29.8	18
	Day 36	$Soil_2$	0.633	**	0.114	0.181	183	16.2	25.4	10

[a] One outlier outside of two standard errors not considered.
[b] Significance levels: $p < 0.1$ (.), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).
[c] Soil temperature in 2 cm depth.
[d] After tillage operations.

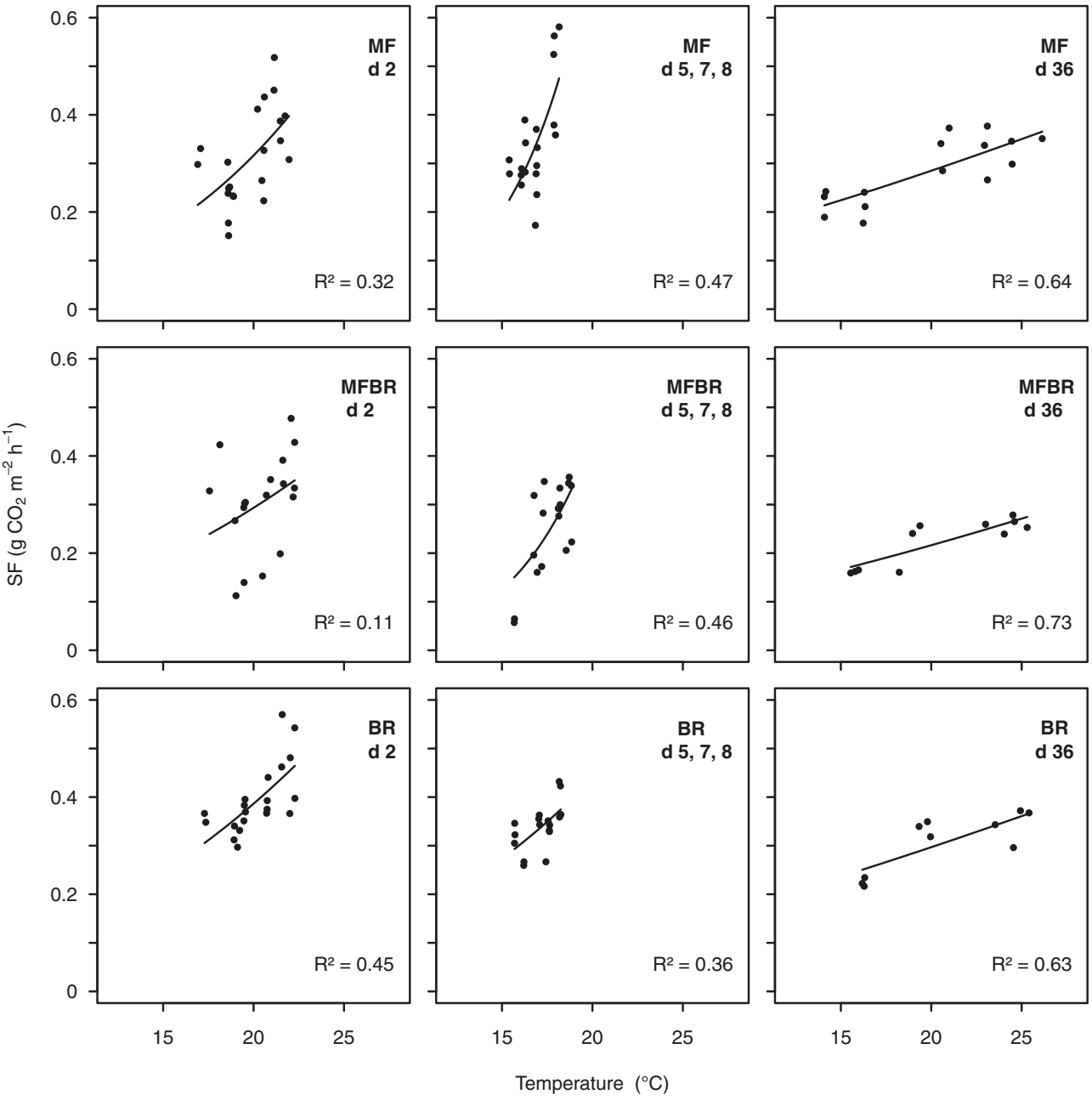

Fig. 4. Temperature driven model fits of soil CO_2 efflux (Eq. (2)) and experimental data for three selected periods of all treatments. Day 36 is assumed to present the bare soil in undisturbed condition. In general, the quality parameters show an increasing tendency with time after tillage, whereas the treatment fertilised with BR was less affected by the management. The depicted temperatures correspond with the selected temperatures in Table 4.

this shift was detectable only on day 36. The activation like parameter (E_0) is a measure for the temperature sensitivity and showed a high variability during the first week for the treatments MF and MFBR. In particular, the period "day 5, 7, 8" showed extraordinarily high E_0 values (>1000 K), whereas after the period "day 7, 8, 9", E_0 values became relatively low and showed little temporal variability. In contrast, the treatment BR did not show such a temporal development of E_0 values. There was only a slight increase in the period "day 4, 5, 7" followed by a slight decrease for the period "day 7, 8, 9". After this, E_0 values became relatively stable as well (Table 4).

The model fits (Eq. (2)) for some of the periods are shown in Fig. 5. The steepness of the fit is a measure for the temperature sensitivity, i.e., the activation like parameter (E_0). The period "day 5, 7, 8" yielded extremely high E_0 values in the treatments MF and MFBR and thus very steep slopes. In contrast, treatment BR did not show such a pattern.

4. Discussion

4.1. Carbon dioxide fluxes

The temperature during this study was in accordance with the long-term observations, but the cumulative precipitation of 35.9 mm during the study period was only around half of the expected amount of 74.2 mm based on long-term data for a 30-year period (1982–2011). Therefore, measured volumetric soil moisture contents were relatively low during the study period (mostly 5–10 vol.%). Since rainfall and therefore soil moisture have a strong impact on soil microbial activity (Franzluebbers et al., 1995;

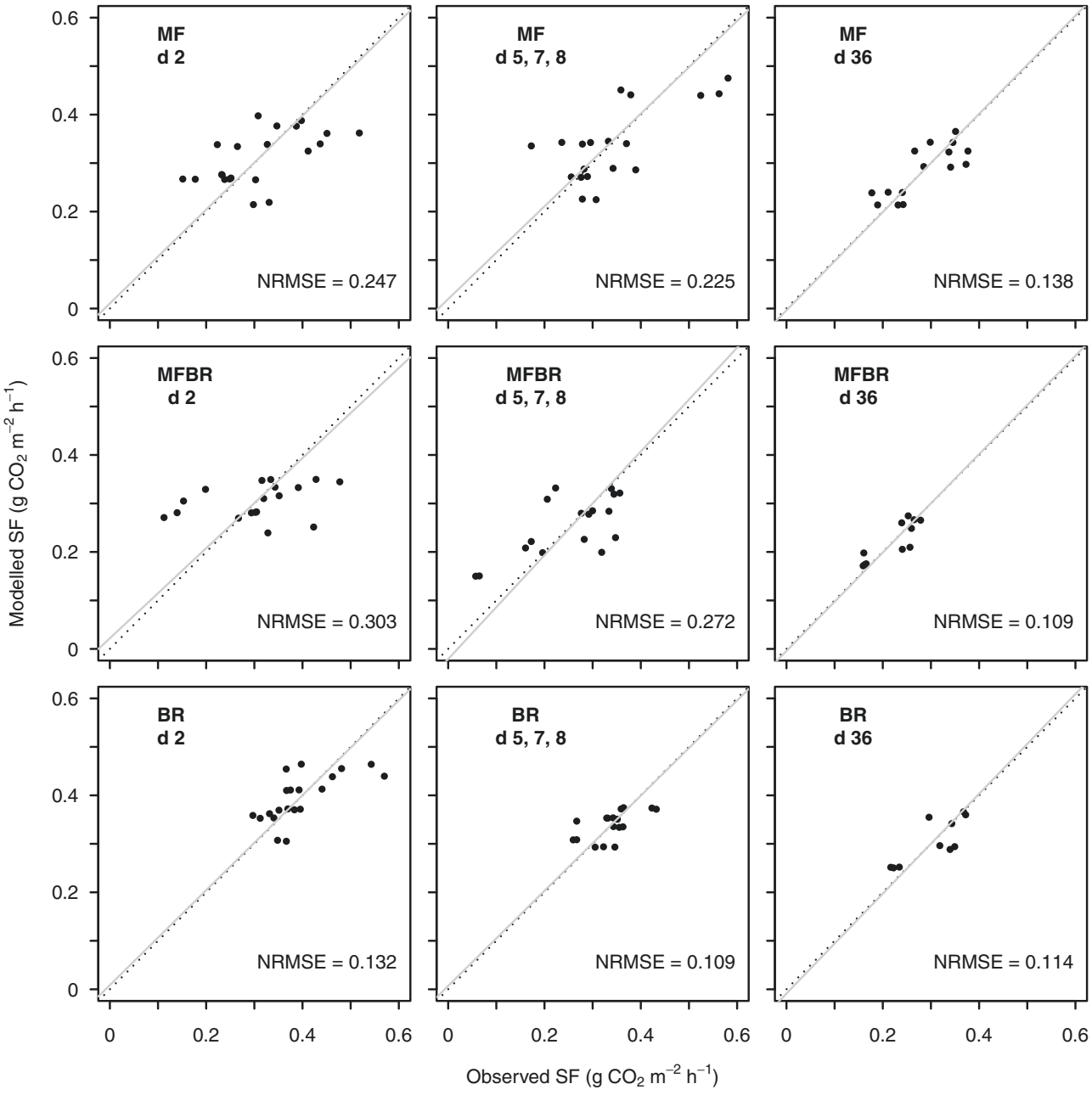

Fig. 5. Observed and modeled soil CO_2 efflux (SF) in selected periods of all treatments. In each plot, the dotted line represents the 1:1 line, whereas the solid line depicts the respective regression line.

Reichstein et al., 2003), SR and CO_2 efflux were probably reduced due to the comparably dry conditions. On the one hand, there was probably less CO_2 in soil pores available for the immediate release after breaking up through tillage (Álvaro-Fuentes et al., 2007). On the other hand, the enhanced turnover of SOC after tillage was maybe limited by low soil moisture (Aslam et al., 2000; Morell et al., 2010; Wang et al., 2000). Actually, water filled pore space after tillage was below 20% in MF and not more than ;1;12 and 14% in BR and MFBR, respectively. Additionally, we tested the correlation between daily mean soil moisture and daily mean CO_2 efflux of each treatment. These correlations proved to be linear for all treatments but were significant ($p < 0.01$, $R^2 = 0.54$) only for the MF treatment. Thus, we would expect higher CO_2 efflux after tillage under "average" conditions because soil moisture can promote the process of CO_2 efflux after tillage (Silva-Olaya et al., 2013).

Average CO_2 efflux directly after tillage operations depended on the extent of soil disturbance and the fertiliser treatment (Fig. 2). Nevertheless, measured fluxes were possibly influenced by liming before tillage (Ahmad et al., 2014; Biasi et al., 2008). In previous studies, similar peaks of this immediate CO_2 efflux as well as the resulting ratios of pre- and post-tillage CO_2 efflux were detected (Table 5). For instance, the investigations by Kessavalou et al. (1998),Al-Kaisi and Yin (2005), Quincke et al. (2007) and Morell et al. (2010) revealed effluxes and post/pre-tillage ratios in accordance with our results. However, higher effluxes and ratios were observed in other studies (Zhang et al., 2011b; Cid et al., 2013), although the mean climatic properties were comparable to

Table 5
Previous studies on the effect of ploughing on CO_2 efflux. Usually, the amount of the peak depended on the magnitude of soil disturbance.

Author (Year)	Experimental design		Results				Site parameters				
	Tillage method[a]	Tillage depth (cm)	CO_2- Peak[b] (g CO_2 m^{-2} h^{-1})	Ratio peak/not tilled	Duration peak (h)	Duration enhanced fluxes (d)	Soil texture	Location	Annual precipitation (mm)	Annual average temperature (°C)	SOC (%)
Akbolat et al. (2009)	MP/CP/RT/FDH	25/35/15/20				33	Clay loam	Turkey	525	12	0.9[d]
Al-Kaisi and Yin (2005)	SC/CP/MP	46/25/25	0.43/0.67/0.79	2.1/3.2/3.8	2	<4	Loam	Iowa, USA			
Alvarez et al. (2001)	MP/DP	15				6 – 13	Sandy loam	Argentina	1020	16.7	1.6
Álvaro-Fuentes (2007)	MP/SC/CP	25–40	0.17 – 13	3 – 15	3		Sandy silt loam	Spain	390 – 475	13.9 – 14.5	
Calderón and Jackson (2002)	RT/FDH		0.04	3	12		Silt loam	California, USA	Summer-dry	Mediterranean-type	0.7
Cid et al. (2013)	SC/DH/C	55/20/25	3.64/5.1/2.71	7.7/10.7/5,7	3	28	Loam	Spain	536	17.6	1.0
Ellert and Janzen (1999)	C	7.5	0.9–1.6	2–4	0.7	1	Loam	Alberta, Canada	400	5.4	1.8[e]
Kessavalou et al. (1998)	MP/C/FDH	15/10/7.5	0.34–0.58	1.7	0.5	<1	Silt loam	Nebraska, USA			
La Scala et al. (2001)	RT/CP/DP/FDH					14	Clay	Brazil	1380	21	1.2
La Scala et al. (2006)	MP + FDH/CP	30/40	2.18/2.41	2/2.2	24	>27	Clay	Brazil	1454	21.6	1.7[d]
Morell et al. (2010)	MP/DP/C	30/30/15	0.15–1.27		2		Clay loam	Spain	430		0.6 – 0.9
Prior et al. (1997)	CP	20	0.23–0.29	1.5–1.6			Loamy sand	Alabama, USA			2.6[d]
Quincke et al. (2007)	DP/CP/MP	10/30/20	0.35–0.46	1.3 – 1.7			Silty clay loam	Nebraska, USA	737	11	1.9
Quincke et al. (2007)	DP/CP/MP	10/30/21	0.58–0.72	2.3–2.7			Silty clay loam	Nebraska, USA	737	11	1.6
Quincke et al. (2007)	DP/CP/MP	10/30/20	0.18–0.61	2.5–8.5			Silty clay loam	Nebraska, USA	737	11	1.5
Reicosky and Lindstrom (1993)	MP/FDH/CP	25/7.5/15	29/7/6[c]	41.4/10/8.6	3		Clay loam	Minnesota, USA			3.1
Reicosky (1997)	MP	22	114[c]	300	3.5		(Silty clay) loam	Minnesota, USA			1.1 – 5.3[d]
Reicosky (1997)	MP	25	49c	42	1		Loam	Minnesota, USA			1.1 – 2.6[d]
Reicosky et al. (1997)	MP/CP	20	16–45[c]	55–83	2		Clay	Texas, USA			1.7 – 3.3
Reicosky (2002)	MP	25	100[c]	111.1	1	87	Loam	Minnesota, USA			2.8 – 3.2[e]
Reicosky and Archer (2007)	MP	10/15/20/28	10–85[c]	21.3–180.8	5	>21	Clay	Minnesota, USA			2 – 3
Rochette and Angers (1999)	MP	20	1.27–3.28	2–5.2	4–8	5–65	Sandy loam	Québec, Canada			2
Silva-Olaya et al. (2013)	FDH	25				20	Clay loam	Brazil	1271	21.5	
Teixeira et al. (2013)	FDH/CP/RT	25/30/8	1.28–1.54	2.9–3.5		15	Clay	Brazil	1380	21	1.8
Teixeira et al. (2013)	MP/CP/RT/SC	30/30/8/35	0.88–0.95	3.4–3.7		16	Clay	Brazil	1380	21	2.3
Willems et al. (2011)	MP		6.91	10.1	9		Fine loamy soil	Ireland	1450	9.6	3.4[e]
Zhang et al. (2011b)	MP	30	2.47–2.81	6.4– .5		>51	Loam	Shaanxi, PR China	584	9.1	1.4[d]

[a] Mouldboard plough (MP), rotary tiller (RT), chisel plough (CP), disc plough (DP), field disc harrow (FDH), subsoil cultivator (SC), cultivator (C).
[b] The term "peak" refers to the initial fluxes of CO_2 directly after tillage.
[c] The relatively high tillage-induced fluxes obtained are accounted for by the use of large chambers (area = 2.71 m^2), where a larger mixing rate inside these chambers may be responsible (Reicosky, 2002; Reicosky et al., 1997).
[d] Derived from SOM values by factor 0.526 (Pribyl, 2010).
[e] Soil total C.

ours. Thus, we assume an influence of soil properties like C content and texture. This is corroborated by investigations on soils with SOC contents up to 5.3% (Reicosky, 1997, 2002; Reicosky and Lindstrom, 1993; Reicosky and Archer, 2007; Reicosky et al., 1997) which showed effluxes higher by orders of magnitude. The duration of the enhanced SR after tillage varied in the previous studies from hours to weeks and months. Although we could not measure an untilled control, we observed a relatively high SD in MF for nearly four weeks (cf. Fig. 2). Compared with the SD before tillage and toward the end of our study, this may indicate the duration of tillage effects on SR. Immediate short-term bursts of physical CO_2 release from soil as a result of accumulated CO_2 in soil pores broken up by tillage are commonly reported (Calderon and Jackson, 2002; Ellert and Janzen, 1999; Reicosky et al., 1997). We observed such a kind of distinct CO_2 burst only in the treatments MF and MFBR for approximately one hour after tillage with the disk harrow. This burst was followed by SR rates that were still higher than before tillage. This prolonged increase in SR is maybe driven by enhanced oxygen availability promoting the microbial activity in the soil, especially in soil aggregates disrupted by the tillage operations (Ellert and Janzen, 1999; Jackson et al., 2003; Reicosky and Archer, 2007). Further, the disruption of soil aggregates supplies microorganisms with organic C which was previously protected, promoting SR (Adu and Oades, 1978; Baldock and Skjemstad, 2000; Six et al., 1999). However, during the first hours, much of the CO_2 release from soil is caused by physical degassing rather than by respiration due to microbial acclimatisation to the altered conditions (Ellert and Janzen, 1999).

In treatment BR, there was also an immediate increase of CO_2 efflux after disking, but no distinct burst. Instead, there was rather a moderate increase followed by a slight decrease of the measured flux over time, similar to the other two treatments after the burst. Probably, the tillage released fewer additional amounts of easily degradable C for microbes. This behavior could be attributed to the fertiliser since BR contains more recalcitrant substrates and exhibit a slower turnover than undigested fertiliser (Bachmann et al., 2014; Chen et al., 2012).

In all treatments, the efflux after the first tillage operation with the deep loosener showed a similar pattern with an immediate increase, but no explicit burst. Since the magnitudes of post-tillage fluxes were higher after disking than after DL, the CO_2 release seems not only to be governed by the depth of soil disturbance (Álvaro-Fuentes et al., 2007; Quincke et al., 2007; Reicosky and Archer, 2007), but also by other factors, like soil comminution. Similarly to our findings, Al-Kaisi and Yin (2005) reported that DL to 46 cm depth had less impact on the initial CO_2 release (with 0.3 g m^{-2} h^{-1}) than chisel or mouldboard ploughing to 25 cm depth (0.5 and 0.6 g m^{-2} h^{-1}, respectively). Recently, such a relationship was also observed by Cid et al. (2013) with 3.6 g CO_2 m^{-2} h^{-1} after DL to 55 cm and 5.1 g after disking to 20 cm soil depth.

Thus, for the increase of CO_2 release the degree of soil comminution seems to play a larger role than the overall depth of the tillage operations. Due to fine comminution by the disking operation, the surface of soil which is affected by the breakdown of aggregates and the disruption of pores is probably larger compared with ploughing. By this means, more CO_2 stored in the soil pore space can physically escape from the soil. Further, more labile C is available to microorganisms for respiration.

The tillage operations on the second day did not cause any additional increase of CO_2 efflux. This can be attributed to the previous physical degassing and the already enhanced respiration induced by the tillage operations the day before. To the contrary, the CO_2 efflux from soil after MP decreased slightly and rose again after drilling. We suggest that the disruption of continuous soil pores through compaction by the packer after MP interrupted the CO_2 efflux, whereas drilling opened them again. In contrast to the other treatments, CO_2 efflux in the BR treatment was only slightly affected by the last two tillage operations. This result could be attributed to the positive effect of organic fertiliser on the soil reaction to compressive stress (Abdollahi et al., 2014). A very similar temporal pattern of CO_2 efflux after multiple tillage operations as in our MF and MFBR treatments was described by Cid et al. (2013), i.e., also no further increase in CO_2 efflux after operations which followed DL and disking.

The order of the treatments in terms of CO_2 efflux after disking on the first day was BR ≈ MF < MFBR. This is an indication of an increased content of stored CO_2 in pores and a potentially higher stock of C available for turnover by microorganism directly after tillage in MFBR treatment (Liu et al., 2006; Ryan and Law, 2005; Uchida et al., 2012).

On the second day, after the end of the tillage operations, the order of CO_2 effluxes changed to MFBR < MF ≈ BR. This finding suggests that the bulk of labile C supply induced immediately by the tillage operations had already been respired in the MFBR treatment. Obviously, the soil microorganisms adapted quickly to the disturbed soil conditions. In general, the microbial structure is able to change relatively fast to adapt to altered conditions in arable soils after tillage (Jackson et al., 2003). Simultaneously, this change is accompanied by an increase of the active microbial biomass (Calderon et al., 2001).

From the third day on, in the treatment MF a relatively high SD was observed. One of the repetitions showed significantly higher fluxes (permutation test, $p < 0.05$) than the others for 27 days. We suppose that the collar of this repetition was placed inadvertently over an accumulation of plant residues buried in the soil. It has been described for other sites that the incorporation of residues increases tillage-induced SR (Alvarez et al., 2001; Dao, 1998; Teixeira et al., 2013). Also in this treatment, precipitation triggered the highest SR of the entire experiment on day 9 since wetting of previously dry soil increases microbial activity and therefore SR in general (Eugster et al., 2010; Kim et al., 2012; Reichstein et al., 2003). Such a phenomenon has been reported also for SR after tillage (La Scala et al., 2006; Silva-Olaya et al., 2013; Zhang et al., 2011a).

4.2. Hot-water extractable SOC

HWC accounts in large parts for microbial biomass and carbohydrates, thus it is recommended as a good indicator for microbial activity (Ghani et al., 2003; Leinweber et al., 1995; Sparling et al., 1998). The observed differences between the treatments in HWC contents are reasonable due to the increasing effect of organic fertilisers on HWC compared with mineral fertilisers (Liang et al., 2012). We think this effect is valid also for digested organic fertiliser like in our study due to comparability of the microbial community composition and microbial activity in soils treated with digested and undigested organic fertiliser (Abubaker et al., 2013; Johansen et al., 2013; Juárez et al., 2013).

The sharp increase of HWC directly after tillage, especially in the two BR treatments, indicates both a formation of labile C and a rapid feedback of microbial community. This behavior could be attributed to a higher proportion of active to dormant micro-organisms in soils fertilised with BR (Odlare et al., 2014). However, this transient change of HWC lasted only a short time, i.e., about one day, and then HWC decreased below the initial contents quickly on the second day again. These observations confirm that the microbial community is able to respond rapidly to disturbances of arable soils (Calderon et al., 2001; Jackson et al., 2003). However, pre-tillage levels of HWC were not attained within the week after tillage, which was maybe due to a small priming effect (Blagodatskaya and Kuzyakov, 2008; De Graaff et al., 2010; Kuzyakov et al., 2000). Overall, the quick decrease of HWC is also

evidence for a limited supply of labile C (Ryan and Law, 2005; Uchida et al., 2012).

4.3. Model evaluation

Due to harvest before tillage, no measurements of undisturbed bare soil were available on the studied site. But the soil was still bare on the last measurement day. Therefore we can assume this last day as a reference for undisturbed soil. In general, all quality parameters of the model fits showed high temporal variability in the days after tillage operations. Directly after tillage, it was impossible to calibrate temperature driven models (Table 4). The relatively narrow temperature range in the noon of "day 2" was the main factor preventing model building. Since a range of 3 °C is deemed to be adequate (Hoffmann et al., 2015), this requirement was met only in treatment MFBR directly after tillage. Especially in the MF treatment, a good model fit was achievable not until day 36. This result can be attributed to the large variability among repetitions in this treatment. Very likely, the tillage induced mineralisation of buried plant residues led to large CO_2 efflux in one repetition for approximately four weeks. In contrast, in the BR treatment a good model fit was attained already from the period "day 13, 15, 27". As the air temperature differed only slightly around a mean of 16.5 °C (SD of 1.1) from day 4 till day 14, the transient and exceptionally good model fit for the period "day 4, 5, 7" indicates a high sensitivity of the disturbed soil to changing environmental parameters (e.g., soil moisture), rather than to temperature (Zhang et al., 2011b). The quality parameters of the treatment MFBR generally ranged between those of the other two treatments, but were insufficient until day 36, similar as in the MF treatment. This could be caused by our approach of pooling the data of measurement days. The daily soil conditions were possibly too different due to the high sensitivity after tillage. However, the model fits of treatment BR recovered relatively fast compared with the other treatments. With regard to the E_0-values, both mineral treatments showed a high variability within the first two weeks. Especially in the period "day 5, 7, 8", remarkably high E_0-values were observed. We suppose a high sensitivity of microbial activity to soil moisture after tillage. Besides temperature, soil moisture and C availability are fundamental for microbial activity and govern the E_0-value (Davidson et al., 2012; Turan et al., 2010). However, the decreasing E_0 values after "day 7, 8, 9" indicate a declining impact of tillage. In contrast, the E_0-values of the treatment BR were relatively stable independently from soil moisture. This indicates a shortage of available C for microbial turnover, since substrate availability plays a crucial role for SR (Kirschbaum, 2006; Liu et al., 2006; Uchida et al., 2012).

Another evidence for the disruption of the temperature dependence of SR by tillage is the shift of the depth where the soil temperatures give a good model fit. For example, in treatment BR there is a change from 10 cm to 2 cm depth after two weeks. We assume that the deeper layers were less affected by tillage and thus yielded a better temperature dependence of SR models. As the effects of tillage faded away with increasing time, the shallower soil layers yielded better model fits again.

5. Conclusions

Tillage has a large influence on subsequent CO_2 efflux patterns and all treatments showed an increased SR after tillage compared to pre-tillage fluxes. Immediately after tillage, mean CO_2 effluxes of up to 0.79 g CO_2 m^{-2} h^{-1} were measured, depending on the extent of soil disturbance and the fertiliser treatment. A burst of physically degassed CO_2 was observed in the treatments MF and MFBR, but not in BR. Disking caused higher CO_2 efflux from soil compared with the other tillage operations, most likely due to fine comminution of the soil. The treatment BR showed a post-tillage pattern different from the other treatments, which can be attributed to the altered soil structure after long-term application of an organic fertiliser like BR. Probably the amount of additionally released labile C from aggregates available for microbes was smaller in BR treatment after tillage than in the others.

Tillage also induced a transient increase of HWC for approximately one day, when the treatment BR showed the highest and MF the lowest change. This quick increase of HWC followed by decrease indicates a fast response of the microbial community to the additional supply with labile C and oxygen after tillage.

Overall, we can state that tillage disturbed the temperature dependence of SR for at least two weeks, with SR being relatively sensitive to environmental parameters like soil moisture rather than temperature. The impact on the recalibration of temperature driven SR models was shorter in treatment BR compared with the MF treatments. On the contrary, an increased mineralisation was observable in treatment MF for four weeks. For this reason, temperature driven models for the prediction of soil derived CO_2 effluxes should be applied carefully for the days and weeks after tillage and verification by measurements in shorter intervals is advisable.

Acknowledgements

We thank the technicians Steffen Kaufmane and Sascha Tittmar for their assistance in our work. Many thanks also to the research facility for agriculture and fisheries of the federal state of Mecklenburg-Western Pomerania (LFA) in Gülzow for their co-operation, especially Jana Peters and Andreas Gurgel. The joint research project underlying this report was funded by the German Federal Ministry of Food, Agriculture and Consumer Protection under the funding identifier 22007910. We thank Ole Wendroth and two anonymous reviewers for their very constructive comments.

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3 Tillage-induced short-term soil organic matter turnover and respiration

SOIL, 2, 475–486, 2016
www.soil-journal.net/2/475/2016/
doi:10.5194/soil-2-475-2016

SOIL Open Access EGU

Tillage-induced short-term soil organic matter turnover and respiration

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Received: 11 December 2015 – Published in SOIL Discuss.: 19 January 2016
Revised: 6 September 2016 – Accepted: 7 September 2016 – Published: 15 September 2016

Abstract. Tillage induces decomposition and mineralisation of soil organic matter (SOM) by the disruption of macroaggregates and may increase soil CO_2 efflux by respiration, but these processes are not well understood at the molecular level. We sampled three treatments (mineral fertiliser: MF; biogas digestate: BD; unfertilised control: CL) of a Stagnic Luvisol a few hours before and directly after tillage as well as 4 days later from a harvested maize field in northern Germany and investigated these samples by means of pyrolysis-field ionisation mass spectrometry (Py-FIMS) and hot-water extraction. Before tillage, the Py-FIMS mass spectra revealed differences in relative ion intensities of MF and CL compared to BD most likely attributable to the cattle manure used for the biogas feedstock and to relative enrichments during anaerobic fermentation. After tillage, the CO_2 effluxes were increased in all treatments, but this increase was less pronounced in BD. We explain this by restricted availability of readily biodegradable carbon compounds and possibly an inhibitory effect of sterols from digestates. Significant changes in SOM composition were observed following tillage. In particular, lignin decomposition and increased proportions of N-containing compounds were detected in BD. In MF, lipid proportions increased at the expense of ammonia, ammonium, carbohydrates and peptides, indicating enhanced microbial activity. SOM composition in CL was unaffected by tillage. Our analyses provide strong evidence for significant short-term SOM changes due to tillage in fertilised soils.

1 Introduction

The influence of tillage on soil organic matter (SOM) is generally well understood. Tillage stimulates decomposition of SOM resulting in increased CO_2 efflux (Dao, 1998), mostly by aeration and by the disruption of macro-aggregates, leading to release of protected SOM (Grandy and Robertson, 2007). In the long term, tillage promotes a shift of chemical structure and age towards more recent SOM (Grandy and Neff, 2008) due to both the mineralisation of older SOM and the decomposition of recent plant residues (Balesdent et al., 1990). In addition, tilled soils contain lower amounts of readily biodegradable (hereinafter referred to as "labile") organic matter (Balota et al., 2003) and have an increased potential for mineralisation and nitrification (Doran, 1980), which implies a lower potential to immobilise mineral N (Follett and Schimel, 1989; Schulten and Hempfling, 1992). However, the immediate, short-term effects of tillage events on SOM are almost unknown.

Research on short-term effects of tillage on SOM has focused largely on CO_2 efflux: several studies have recorded the dynamics of CO_2 efflux immediately after tillage (see Table 5 in Fiedler et al., 2015) and some basic models have been developed that describe correlations between CO_2 efflux and the turnover of soil organic carbon (SOC) after tillage by first-order kinetics (La Scala et al., 2008). Admittedly, these correlations do not causally explain which organic components are mineralised. Furthermore, SOM–CO_2 efflux relationships are influenced by the type of soil amendment (Fiedler et al., 2015).

Biogas digestate is a relatively new type of soil amendment, and its long-term stability in soil is still under debate, as recently reviewed by Möller (2015). Consequently, it is not clear how long-term application of biogas digestates would alter the composition of SOM, and tillage effects on short-term SOM turnover in biogas digestate-amended soils are almost unstudied. Even short-term changes in SOM may have strong effects on nutrient availability and plant productivity. A better understanding of the immediate impacts of tillage on SOM and its turnover may help to avoid adverse effects for plant growth (Franzluebbers et al., 1994).

In general, detecting changes in the molecular-chemical composition of SOM on timescales as short as days requires extremely sensitive methods. Pyrolysis-field ionisation mass spectrometry (Py-FIMS) is a very sensitive method and has been applied successfully to investigate differences in the chemical composition of SOM under different fertiliser treatments like mineral NPK fertiliser or farmyard manure (Jandl et al., 2004; Leinweber et al., 2008; Schmidt et al., 2000). Even very small alterations in the composition and stability of dissolved organic matter – a very reactive part of SOM – during storage in the refrigerator (Schulten et al., 2008) or diurnal cycles of CO_2 assimilation and respiration (Kuzyakov et al., 2003; Melnitchouck et al., 2005) have been detected and resolved by multivariate statistics of mass-spectrometric fingerprints. Furthermore, Py-FIMS of bulk SOM has revealed alterations in laboratory incubation experiments and allowed for these to be linked to respiration and enzyme activities (Leinweber et al., 2008). However, it is unclear whether the method is sensitive enough to detect tillage-induced SOM alterations under various fertilisation regimes and analyse its influence on CO_2 efflux at the field scale, where spatial heterogeneity may interfere with the temporal dynamics much more than in the above-cited laboratory studies.

Hot-water extraction is a relatively simple method to release labile SOM and to estimate how much of soil C and N can be easily utilised by microorganisms (Leinweber et al., 1995). These labile pools have been suggested to be an important indicator of short-term changes in SOM quality due to soil management (Haynes, 2005). Furthermore, a significant proportion of hot-water-extracted organic matter originates from microbial biomass. Thus, this approach is a potential indicator for changes in microbial biomass or activity (Sparling et al., 1998), which may reflect sources of CO_2 efflux following tillage.

Here, we investigate (1) short-term effects of tillage on SOM composition and (2) potential relationships between decomposable SOM fractions and measured CO_2 efflux under the impact of different soil amendments by combining Py-FIMS with CO_2 efflux measurements.

2 Materials and methods

2.1 Study site

The study site is located in northeastern Germany in the ground moraine of the Weichselian glacial period at 53°48′35″ N, 12°4′20″ E (elevation 10 m) within a gently rolling relief. The soil is a Stagnic Luvisol (IUSS Working Group WRB, 2006) with sandy loam texture (sand: 63 %; silt: 26 %; clay: 11 %) overlying bedrock of till. The topsoil (0–30 cm) has an organic carbon content of 8.5 ± 0.2 mg g^{-1} (mean ± standard deviation, $n = 9$), pH of 7.4 ± 0.9 ($n = 3$) and bulk density of 1.51 ± 0.08 g cm^{-3} ($n = 3$), measured according to Fiedler et al. (2015). The climate is characterised by maritime influence, with annual averages of 8.8 °C temperature and 557 mm total precipitation for the 30-year period from 1985 to 2014 (Federal State Research Institute for Agriculture and Fisheries Mecklenburg-Western Pomerania (LFA), Germany, personal communication, 2015). The experiment was conducted on a field which has been cultivated with maize (*Zea Mays* L. 'Atletico') as feedstock for a biogas plant. Before our study period, during other trials, winter wheat (*Triticum aestivum* L.) and subsequently maize were grown on the field.

We compared three fertiliser treatments: CL – without fertiliser (control); MF – with mineral fertiliser; and BD – with biogas digestate. The size of the three experimental plots was 6 m × 30 m each. In both fertilised treatments, equal overall amounts of plant-available N were applied (160 kg ha^{-1}) on 26 April 2012. The mineral fertiliser calcium ammonium nitrate was top-dressed, whereas the biogas digestate was injected into the soil down to 10 cm depth with a track width of 25 cm. Following recommendations of the LFA (personal communication, 2012), a mineral fertiliser equivalent of 70 % of total N in the biogas digestates (229 kg N ha^{-1}) was assumed. The digestate for this single application originated from anaerobic fermentation of 91 % cattle slurry, 7 % rye groats and 2 % maize silage; it had a pH of 8.1 and 3.8 % C, 0.5 % total N and 0.3 % NH_4-N in undried material. During the cropping season 2012, maize was grown according to conventional agricultural practice.

Sixteen days after harvest of the maize (8 October 2012), the field site was first tilled with a Väderstad Carrier 300 disc harrow down to 10 cm depth (24 October, about 09:15 LT) and then with a Överum CX 490 reversible mouldboard plough down to 30 cm depth on the subsequent day (25 October, about 11:30 LT).

2.2 CO_2 concentration measurement and estimation of CO_2 efflux

For measuring CO_2 exchange, we permanently installed three replicate bases in each treatment after fertilisation in spring, which were removed for tillage and inserted back afterwards. The adjacent bases were placed 1 m apart. The

bases had dimensions of 79 cm × 79 cm and a total height of 15 cm and were installed into the soil down to 12 cm depth. The CO_2 concentration measurements where performed with two LI-COR (Lincoln, NE, USA) LI-820 infrared gas analysers, each connected to a non-steady-state closed chamber that was placed on the bases during measurements. The chambers had an area of 0.6241 m^2 and a height of 0.55 m, resulting in a chamber volume of 0.34 m^3, and were equipped with small fans (80 mm × 80 mm × 25 mm, 3000 rpm, 68 $m^3\,h^{-1}$) in order to mix and homogenise the air inside the chambers. Due to the successive measurement of the replicate bases in each treatment, we obtained pseudo-replications.

During chamber placement, we recorded CO_2 concentrations in the chamber headspace with 1.3 s intervals for 3 to 5 min, resulting in approximately 140 to 230 data points per measurement. Fluxes were estimated with the function"fluxx" of the package "flux" version 0.3-0 (Jurasinski et al., 2014) for the R statistical software version 2.15.2 (R Core Team, 2013). In short, the algorithm identifies the most linear part of the CO_2 concentration development during chamber placement time and fits a linear regression model (Eq. 1):

$$f = \frac{MpV}{RTA}\frac{\mathrm{d}c}{\mathrm{d}t}10^6, \quad (1)$$

with f the CO_2 flux ($g\,m^{-2}\,h^{-1}$), M the molar mass of CO_2 ($g\,mol^{-1}$), p the air pressure (Pa), V the chamber volume (m^3), R the gas constant ($J\,mol^{-1}\,K^{-1}$), T the temperature inside the chamber (K), A the area covered by the chamber (m^2), and $\mathrm{d}c/\mathrm{d}t$ the CO_2 concentration change over time ($ppm\,h^{-1}$). The minimum proportion of data points to be kept for regression analyses was 70 % of a concentration measurement. This allowed for discarding of data noise at the beginning and the end resulting from chamber deployment and removal (for details, see the help file for the function "fluxx" of the package "flux"). Thus, each CO_2 flux was estimated from at least 98 concentration measurements. Only linear fluxes with a concentration change of at least 10 ppm, a normalised root-mean-square error (NRMSE) ≤ 0.15 and a coefficient of determination (R^2) of at least 0.85 were included in further analyses. We assumed linearity of concentration change and did not test for non-linearity since 95.1 % of the obtained linear regressions had $R^2 \geq 0.95$.

To obtain reference data from before tillage operations, the undisturbed site was measured hourly between 07:00 and 13:00 LT on 19 October 2012 (i.e. between harvest and tillage). The intervals between measurements before, during and after tillage operations were varied to effectively capture the development of CO_2. The measurements immediately after the tillage operations were conducted within 1 min by inserting the collars and putting on the airtight chambers. The timeline (24 until 29 October) of tillage events, soil samplings and the respective CO_2 measurements, together with soil temperature, is shown in Fig. 1. After this period, CO_2 measurements were performed hourly before noon on 1, 5 and 9 November.

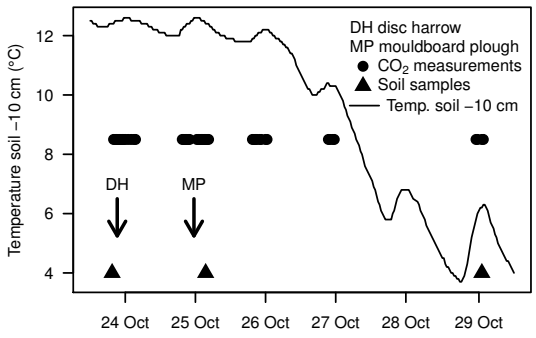

Figure 1. Timeline of the soil sampling and the CO_2 measurements in relation to the tillage events. Additionally, soil temperature at 10 cm depth, which was recorded every 30 min with an automated meteorological station (DALOS 535, F & C, Gülzow, Germany), is plotted.

2.3 Soil sampling and analyses

Three replicates of bulk soil samples were taken between 0 and 10 cm depth (depending on unevenness of soil surface due to tillage) directly with three soil sample rings ($h = 6.1$ cm, $V = 250\,cm^3$) in a triangular arrangement around the three bases for gas sampling (see Sect. 2.2) in each treatment at three times: (1) right before the first tillage operation, (2) in the afternoon after the second tillage operation and (3) 4 days after the second tillage operation. The resulting 27 soil samples were fixed immediately with liquid nitrogen and split thereafter into subsamples for freeze drying and for oven drying at 60 °C.

For Py-FIMS, the freeze-dried samples were finally ground and homogenised using a planetary ball mill. Then, about 2 g of each sample was transferred into a Petri dish with a spatula and three crucibles were filled by drawing them across the dishes. These subsamples of about 5 mg were thermally degraded in the ion source (emitter: 4.7 kV; counter electrode −5.5 kV) of a double-focusing Finnigan MAT 95 mass spectrometer (Finnigan, Bremen, Germany). The samples were heated in a vacuum of 10^{-4} Pa from 50 to 700 °C, in temperature steps of 10 °C over a time period of 15 min. Between magnetic scans the emitter was flash heated to avoid residues of pyrolysis products. The Py-FIMS mass spectra of each sample were gained by the integration of 65 single scans in a mass range of 15–900 *m/z*. Ion intensities were referred to 1 mg of the sample. Volatile matter was calculated as mass loss in percentage of sample weight. For plotting, the three replicates of each sample were then averaged to one final survey spectrum. Moreover, thermograms were compiled for the total ion intensities. The assignment of marker signals to chemical compounds from the survey spectra were interpreted according to Leinweber et al. (2013) to obtain the

relative abundance of ten SOM compound classes: (1) carbohydrates, (2) phenols and lignin monomers, (3) lignin dimers, (4) lipids, alkanes, alkenes, bound fatty acids and alkyl monoesters, (5) alkyl aromatics, (6) mainly heterocyclic N-containing compounds, (7) sterols, (8) peptides, (9) suberin, and (10) free fatty acids.

Subsamples of oven-dried and sieved soil (2 mm) were used for determination of total and hot-water-extracted C and N. For determination of total C and N, 1 g of ground soil was analysed with a vario Max CN elemental analyser (elementar Analysensysteme GmbH, Hanau, Germany) based on high-temperature combustion at up to 1200 °C with subsequent gas analysis. For hot-water extraction, 20 g of soil was boiled in 40 mL of deionised water for 60 min (Leinweber et al., 1995). After filtration with pleated filters (240 mm, 80 g m^{-2}) by Munktell (Falun, Sweden), extracts were analysed with a DIMATOC 2000 (DIMATEC Analysentechnik GmbH, Essen, Germany) for determination of hot-water-extractable organic C (HWC) as well as organic and inorganic bound N, often referred to as "total nitrogen bound" (HWN). These measurements of organic C and total nitrogen bound are based on the principle of thermal–catalytic oxidation with subsequent NDIR detection and the principle of chemiluminescence, respectively. For each sample, two replicates were analysed and results were averaged for further calculations.

2.4 Statistical analyses

All statistical analyses were run using R 2.15.2 (R Core Team, 2013). The cumulated CO_2 effluxes were estimated by a bootstrap method with the function "auc.mc" of the R package "flux" version 0.3-0 (Jurasinski et al., 2014). In detail, the CO_2 fluxes were cumulated in 250 iterations, while for each run 25 fluxes were omitted randomly for the period after tillage. For the reference period before tillage, in each iteration run, four fluxes were omitted randomly. The numbers of randomly omitted fluxes per run correspond to roughly one-fifth of the recorded fluxes per treatment in the respective periods. The resulting data were used to calculate means and standard deviations. Tukey's HSD test was applied to test for differences in means of CO_2 fluxes as well as HWC and HWN between sampling periods and treatments against a significance level of $\alpha < 0.05$. Py-FIMS signals of the compound classes were tested for differences in means by Tukey's HSD test against a significance level of $\alpha < 0.1$ since the number of replicates was limited and the variances were rather high.

A principal component analysis (PCA) was applied to the mass signals with significant differences between the samples according to the univariate Wilks' λ ($p < 0.001$) with function "rda" of R package "vegan" version 2.3-0 (Oksanen et al., 2015).

Partial least-squares regression (PLSR) was used for discrimination (Barker and Rayens, 2003) to explore linkages between shifts in the *m/z* data by tillage and shifts in CO_2 efflux. PLSR models were built using function "autopls" of the R package "autopls" version 1.3 (Schmidtlein et al., 2015) with stepwise backward selection combined with a 10-fold cross-validation to substantially reduce the number of variables, i.e. to extract the variables with the highest explanatory power. The PLSR procedure was repeated 10 000 times to yield coherent results since the obtained PLSR models differed widely both in the number and in the choice of variables and thus in their predictive performance. Based on the performance index suggested by Bauwe et al. (2015), the 500 "best" models were obtained and, finally, the mass signals which were utilised more than 50 times in the latter models were extracted.

3 Results

3.1 Soil organic carbon, nitrogen, hot-water-extractable carbon and hot-water-extractable nitrogen

One of the replicates in MF exhibited exceptionally low HWC and HWN values. According to Dixon's Q test, these values were outliers (one-third and one-half, respectively, as high as for the other replicates in MF) and thus excluded from further analysis. Before tillage, the soil of all treatments had similar C and HWC contents, but differences appeared between MF and BD, where the N and HWN contents were slightly higher in MF, resulting in narrower C / N and HWC / HWN ratios in MF (8.5 and 5.9, respectively) compared to BD (9.0 and 8.5, respectively) (Table 1). The C, N and HWC contents of all treatments changed only slightly after tillage, but the HWN content of soil in BD increased significantly ($p < 0.05$) from 0.05 mg g^{-1} (5.6 % of N) to 0.07 mg g^{-1} (7.4 % of N), resulting in a significant ($p < 0.05$) narrowing of the HWC / HWN ratio from 8.5 to 6.0 (Table 1).

3.2 Soil CO_2 efflux

Five days before the tillage operations (19 October 2012), the mean efflux rates (all in g CO_2-C m^{-2} h^{-1}) were 0.133 (CL), 0.192 (MF) and 0.173 (BD), with the efflux being significantly lower from CL than from the amended plots MF and BD ($p < 0.05$) (Fig. 2). In the morning before the first tillage operation with a disc harrow (24 October), the effluxes had similar magnitudes and proportions to 5 days before (CL = 0.147, MF = BD = 0.199, all in g CO_2-C m^{-2} h^{-1}). After harrowing, CO_2 effluxes increased to 0.849 (CL), 0.833 (MF) and 0.479 (BD). Over the next 5.5 h, these values declined to 0.602 (CL), 0.460 (MF) and 0.276 (BD), resulting in overall mean effluxes of 0.554 (CL), 0.481 (MF) and 0.344 (BD), with the latter being now significantly lower ($p < 0.05$) than CL or MF during the measured period after harrowing. Directly before the second tillage operation with a reversible mouldboard plough in the morning of the following day (25 October), the mean effluxes were 0.299 (CL),

Table 1. Means and standard deviations of soil organic carbon (C), nitrogen (N), C / N ratio, hot-water-extractable carbon (HWC) and nitrogen (HWN) and HWC / HWN ratio before (Pre), after (Post) and 4 days after tillage (Post + 4). Different letters ([a], [b]) in each column within treatments (BD, biogas digestate; MF, mineral fertiliser; CL, control) indicate significant differences (Tukey's HSD test, $p < 0.05$) in means.

Treatment	Date	C (mg g^{-1})	N (mg g^{-1})	C / N	HWC (mg g^{-1})	HWN (mg g^{-1})	HWC / HWN
BD	Pre	8.4 ± 0.1	0.9 ± 0.0	9.0 ± 0.1	0.44 ± 0.02	0.05 ± 0.00[a]	8.5 ± 0.1[a]
	Post	8.5 ± 0.1	1.0 ± 0.0	8.8 ± 0.3	0.44 ± 0.03	0.07 ± 0.01[b]	6.1 ± 0.4[b]
	Post + 4	8.4 ± 0.6	1.0 ± 0.0	8.7 ± 0.0	0.40 ± 0.02	0.07 ± 0.01[b]	6.0 ± 0.4[b]
MF	Pre	8.7 ± 0.3	1.0 ± 0.0	8.5 ± 0.2	0.44 ± 0.05	0.08 ± 0.00	5.9 ± 0.8
	Post	8.4 ± 0.3	1.0 ± 0.0	8.5 ± 0.1	0.42 ± 0.04	0.09 ± 0.02	4.9 ± 0.7
	Post + 4	8.6 ± 0.2	1.0 ± 0.0	8.5 ± 0.1	0.31 ± 0.14	0.06 ± 0.01	5.0 ± 0.8
CL	Pre	8.5 ± 0.2	1.0 ± 0.0	8.8 ± 0.2	0.50 ± 0.10	0.06 ± 0.02	8.9 ± 1.3
	Post	8.6 ± 0.2	1.0 ± 0.0	8.8 ± 0.0	0.48 ± 0.04	0.05 ± 0.01	8.8 ± 0.8
	Post + 4	8.5 ± 0.0	1.0 ± 0.0	8.7 ± 0.1	0.40 ± 0.03	0.04 ± 0.00	9.6 ± 0.3

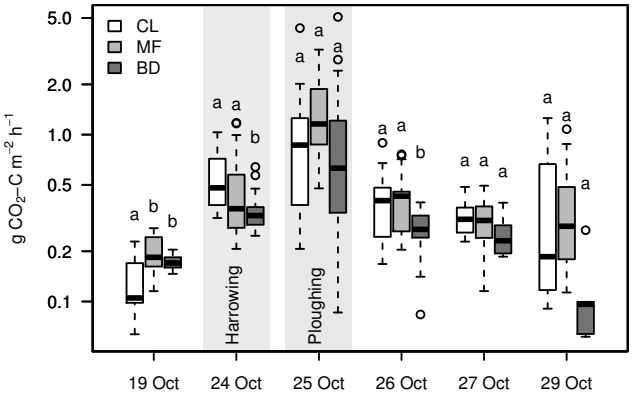

Figure 2. Soil CO_2 efflux and time of tillage operations (harrowing down to 10 cm depth and ploughing down to 30 cm depth). Note that, for the days of tillage (24 and 25 October), only the fluxes after tillage (distinguished by light-grey backgrounds) are included in order to gain a better attribution of the tillage effect. Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$) in mean fluxes of the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) for each measurement day.

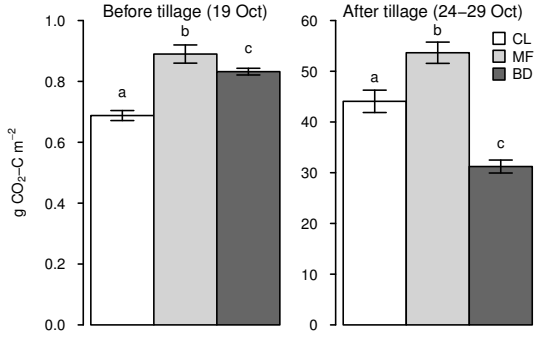

Figure 3. Cumulated soil CO_2 effluxes on a day before (19 October, between 07:00 and 13:00 LT) and the period (24 October, 07:00 LT–29 October, 13:00 LT) tillage. Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$) in means of the cumulated fluxes of the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) before and after. Error bars represent the standard deviation of interpolation by bootstrapping after 250 iteration runs.

0.249 (MF) and 0.290 (BD) (all in g CO_2-C m^{-2} h^{-1}). Immediately after ploughing, they increased sharply up to 2.443 (CL), 2.654 (MF) and 3.347 (BD) and declined to 0.371 (CL), 0.718 (MF) and 0.223 (BD) after 4 h, leading to overall mean effluxes of the measured period after ploughing of CL = 1.012, MF = 1.392, and BD = 1.020. Although the mean CO_2 fluxes within each treatment differed significantly ($p < 0.05$) from the other measured days only after ploughing (25 October), BD on average showed significantly ($p < 0.05$) lower fluxes than CL or MF after tillage on 24 and 29 October (Fig. 3), as well as on 1 November (CL = 0.262, MF = 0.242, BD = 0.113, all in g CO_2-C m^{-2} h^{-1}) and 5 November (CL = 0.331, MF = 0.316, BD = 0.074, all in g CO_2-C m^{-2} h^{-1}).

3.3 Pyrolysis-field ionisation mass spectrometry

The thermograms of total ion intensity (TII) and the Py-FIMS mass spectra of the soil samples of CL and MF taken before tillage were similar, whereas the ones of BD differed markedly from those two (Fig. 4): the TII thermograms of CL and MF had a peak at 480 °C, but BD displayed a pronounced bimodal shape with a first volatilisation maximum at about 390 °C which was less marked in CL and MF. Furthermore, the mass spectrum of BD differed distinctly from the mass spectra of MF and CL; in particular, the abundance of marker signals for carbohydrates and peptides (e.g. *m/z* 58, 60, 84, 69, 110, 126 and 162) was lower. Apart from this the spectra are dominated by signals for lignin monomers and dimers (e.g. *m/z* 150, 208, 222, 244) as well as for homologous series of alkenes and alkadienes from *n*-C_{18} up (e.g. *m/z* 252, 264/266, 278/280, 294, 308, 322, 336, 364, 392, 406) (Fig. 4).

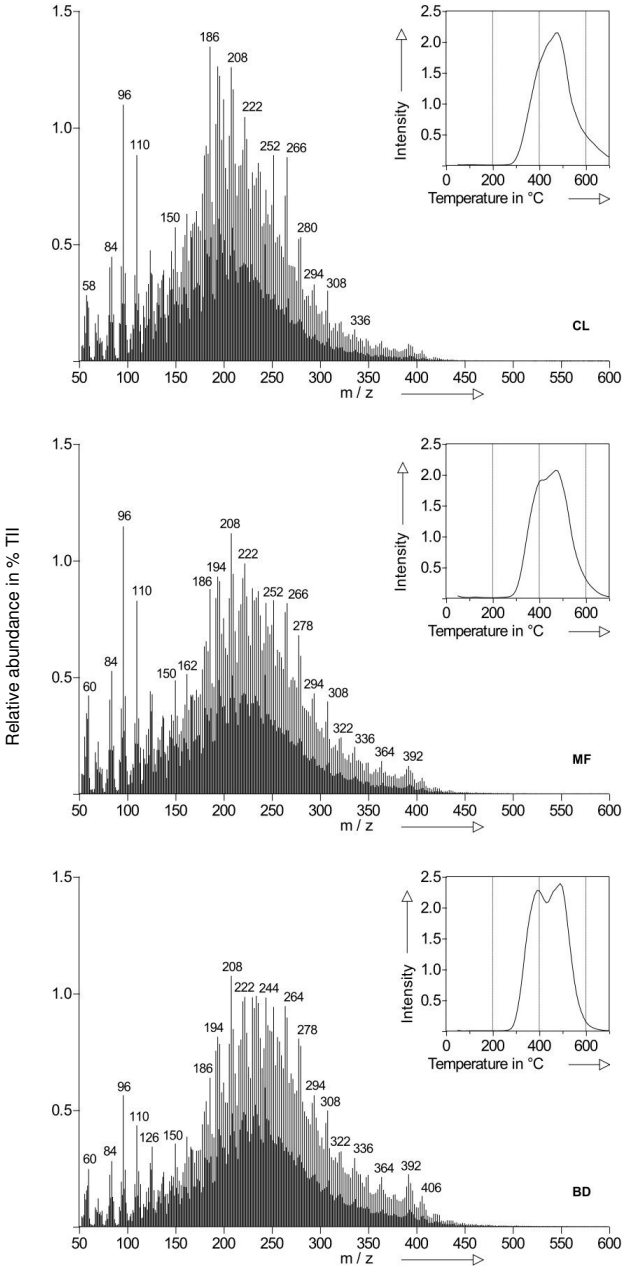

Figure 4. Thermograms of total ion intensity (TII, upper right insets) and summed pyrolysis-field ionisation mass spectra of the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) before tillage.

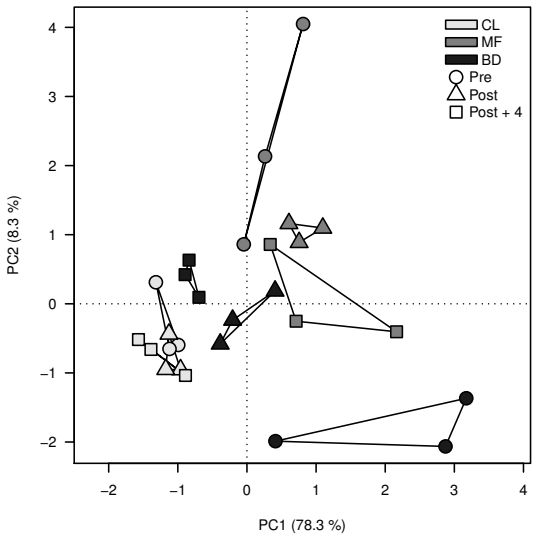

Figure 5. Principal component analysis of mass signals with significant differences according to Wilks' λ. Treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) and sampling times (pre-tillage, post-tillage and post-tillage + 4 days) are depicted by different colours and symbols, respectively. Since the areas integrated by the corresponding three sampling points do not overlap for the fertilised treatments, significant distinctions and changes in relative SOM composition can be assumed before and after tillage, respectively.

After discriminant function analysis with Wilks' λ, the resulting significant relative mass signals ($p < 0.001$, $n = 67$) were further explored by PCA. The first two principal components accounted for 78.3 and 8.3 % of total variance. All treatments are well separated from each other (Fig. 5), with CL mainly in the third quadrant, MF mainly in the first quadrant and BD spanning from the second to the fourth quadrant. According to this analysis, samples from MF and BD taken before the tillage events (Pre) showed the largest differences in composition. The PCA separated the samples taken at different dates (Pre, Post and Post + 4) in the treatments MF and BD, but not in CL.

Basic data of the Py-FI mass spectra and the proportions of compound classes are compiled in Table 2. Approximately 46.9 % of the TII in the mass spectra could be explained by *m/z* signals assigned to the compound classes. Additionally, non-specific low-mass signals and isotope peaks contributed 2.6 and 14.2 %, respectively. Before tillage, the volatilised matter (VM) was highest in BD and increased from 5.2 to 7.1 % during the days after tillage. Such an increase over time was only observed for BD, but it was not significant ($p > 0.1$). In the other treatments, a temporal increase in VM occurred directly after the first tillage with disc harrow.

The relative (Table 2) and absolute (data not shown) ion intensities of the compound classes varied across treatments before tillage and changed differently after tillage. In the undisturbed soil, BD had the lowest proportions of carbohydrates, heterocyclic N-containing compounds and peptides and the highest proportions of lignin dimers, lipids, sterols, suberin and free fatty acids. CL was characterised by higher proportions of phenols and lignin monomers, whereas MF ranged between BD and CL regarding the proportions of these compound classes. In BD, the relative proportions of the samples taken after tillage displayed significant ($p < 0.1$) increases in carbohydrates, phenols and lignin monomers,

Table 2. Total ion intensity (TII), percentage of matter volatilised in pyrolysis (VM), and relative contribution of soil organic matter compound classes to the TII as detected by Py-FIMS in the treatments (CL, control; MF, mineral fertiliser; BD, biogas digestate) before (Pre), after (Post) and 4 days after tillage (Post + 4) with standard deviations. Different letters (^{a}, ^{b}) in a column within each treatment indicate significant differences (Tukey's HSD test, $p < 0.1$) in means of the different dates towards tillage. Additionally, treatments with significant changes are highlighted in bold.

Treatment	Date	TII	VM (%)	Relative proportions of compound classes (% TII)*									
		(10^6 counts mg^{-1})		CHYDR	PHLM	LDIM	LIPID	ALKYL	NCOMP	STEROL	PEPTI	SUBER	FATTY	Sum
BD	Pre	44.3 ± 11.5	5.2 ± 1.3	**3.7 ± 1.8**^{a}	**9.8 ± 3.8**^{a}	3.4 ± 1.4	**5.3 ± 1.0**^{a}	11.9 ± 1.2	**1.8 ± 0.8**^{a}	**1.6 ± 0.7**^{a}	**4.3 ± 1.2**^{a}	0.1 ± 0.1	**0.5 ± 0.2**^{a}	**42.3 ± 5.4**^{a}
	Post	40.3 ± 19.3	4.7 ± 1.3	**5.6 ± 0.3**^{a,b}	**13.3 ± 0.8**^{a,b}	2.5 ± 0.4	**4.1 ± 0.1**^{b}	12.5 ± 0.7	**2.8 ± 0.2**^{b}	**0.7 ± 0.2**^{b}	**5.5 ± 0.3**^{a,b}	0 ± 0.1	**0.2 ± 0.1**^{b}	**47.3 ± 0.9**^{a,b}
	Post + 4	35.1 ± 3.0	7.1 ± 1.2	**6.2 ± 0.3**^{b}	**14.4 ± 0.3**^{b}	1.9 ± 0.2	**3.9 ± 0.1**^{b}	13.2 ± 0.1	**3.2 ± 0.2**^{b}	**0.6 ± 0**^{b}	**5.9 ± 0.2**^{b}	0 ± 0	**0.2 ± 0**^{b}	**49.4 ± 0.7**^{b}
MF	Pre	34.2 ± 3.4	3.9 ± 1.1	5.6 ± 0.9	11.4 ± 0.7	2.9 ± 0.4	**4.6 ± 0.4**^{a}	12.2 ± 0.9	2.7 ± 0.2	1 ± 0.4	5.4 ± 0.7	0 ± 0	0.3 ± 0.3	46.0 ± 0.3
	Post	39.1 ± 5.2	4.6 ± 1.0	4.6 ± 0.2	10.5 ± 0.6	3.5 ± 0.2	**5.1 ± 0.1**^{a,b}	12.4 ± 0.3	2.3 ± 0.1	1.2 ± 0.2	4.8 ± 0.2	0 ± 0	0.1 ± 0.1	44.5 ± 0.8
	Post + 4	46.5 ± 15.8	4.2 ± 0.5	4.3 ± 1.0	10.3 ± 1.6	3.3 ± 0.5	**5.4 ± 0.4**^{b}	12.6 ± 0.5	2.2 ± 0.5	1.2 ± 0.3	4.5 ± 0.4	0 ± 0.1	0.3 ± 0.1	44.2 ± 2.8
CL	Pre	41.5 ± 15.5	**3.6 ± 0.6**^{a}	5.5 ± 0.3	14.3 ± 0.4	2.2 ± 0.8	4.3 ± 0.1	13.6 ± 0.4	3.1 ± 0.2	0.6 ± 0	5.4 ± 0.2	0 ± 0	0.2 ± 0.2	49.2 ± 0.9
	Post	41.2 ± 7.8	**4.7 ± 0.4**^{b}	5.6 ± 0.3	14.4 ± 0.2	1.8 ± 0.1	4.5 ± 0.2	13.9 ± 0.1	3.1 ± 0.1	0.6 ± 0.1	5.4 ± 0.3	0 ± 0	0.3 ± 0.1	49.6 ± 0.6
	Post + 4	47.9 ± 14.8	**3.2 ± 0.5**^{a}	5.6 ± 0.5	14.4 ± 0.6	2.5 ± 0.8	4.3 ± 0	13.7 ± 0.5	3.1 ± 0.2	0.6 ± 0.1	5.3 ± 0.2	0 ± 0	0.1 ± 0.1	49.5 ± 1.3

* CHYDR, carbohydrates with pentose and hexose subunits; PHLM, phenols and lignin monomers; LDIM, lignin dimers; LIPID, lipids, alkanes, alkenes, bound fatty acids, and alkyl monoesters; ALKY, alkyl aromatics; NCOMP, mainly heterocyclic N-containing compounds; STEROL, sterols; PEPTI, peptides; SUBER, suberin; FATTY, free fatty acids.

alkyl aromatics, heterocyclic N-containing compounds and peptides, while lignin dimers, lipids, sterols and free fatty acids decreased. In MF, the proportion of lipids increased, while carbohydrates and peptides decreased. No changes were detected in the unfertilised treatment (CL). The discrimination of relative mass signals with PLSR to explain cumulated CO_2 efflux revealed mainly functional groups from ketones and amides, peptides, carbohydrates, and lignin building blocks and fatty acids (Table 3).

Linear correlations were calculated to check relationships between HWC, HWN and soil respiration as indicators of SOM dynamics (Kuzyakov, 2006; Leinweber et al., 1995) and the absolute signal counts of the compound classes (Fig. 6). The latter was derived from Table 2 by Eq. (2).

$$\mathrm{CII_{abs}} = \frac{\mathrm{TII} \times \mathrm{CII_{rel}}}{100}, \tag{2}$$

with $\mathrm{CII_{abs}}$ the absolute ion intensity of the respective compound class, TII the total ion intensity and $\mathrm{CII_{rel}}$ the proportion of the ion intensity of the respective compound class.

In MF, the ion intensities for carbohydrates were positively correlated with HWC ($R^2 = 0.44$), whereas, in contrast, no such a correlation was found in BD. However, HWN showed a positive correlation with carbohydrates in BD ($R^2 = 0.61$). Further, CO_2 efflux increased with decreasing amounts of sterols in BD ($^2 = 0.40$).

4 Discussion

4.1 Bulk soil and hot-water-extracted carbon and nitrogen

The C, HWC, N and HWN contents of the treatments showed no differences before tillage (Table 1), thus confirming the outcomes of other field experiments with similar fertilisers (Makádi et al., 2016; Odlare et al., 2014). However, the C and N contents obtained may not be representative of long-term effects of biogas digestate vs. mineral fertiliser, which may also depend on soil texture (Makádi et al., 2016).

The increase in HWN in BD after tillage indicates an increase in easily mineralisable organic N which probably originates from soil biomass and lysates (Ghani et al., 2003; Leinweber et al., 1995) and implies an accelerated microbial turnover of soil organic N. This seems reasonable since the microbial community is able to adjust its structure and activity relatively fast to utilise formerly protected organic matter after exposure due to disruption of aggregates by tillage (Jackson et al., 2003; La Scala et al., 2008). Accordingly, Fiedler et al. (2015) observed a short-lived increase in HWC after the first of 2 days of several tillage operations which was not found in the present study. It is possible that we did not detect it because we did not take any soil samples after the first day. Overall, a single amendment with biogas digestates is very likely insufficient to initiate changes in bulk soil C and N levels. However, the increased HWN levels in BD

Table 3. Results of iterative partial least-squares regression for cumulated CO_2 efflux as an dependent variable and *m/z* data of all treatments and sampling times as explaining variables.

m/z	Molecule/compound class
17/18	Ammonia/ammonium
31	$[M+H]^+$ of formaldehyde
34	H_2S
43	C_2H_3O from ketones/amides and C_3H_7 propyl
46	Formic acid
55	C_3H_3O from ketones/amides
57	C_3H_5O from ketones/amides and C_4H_9 butyl
73	Propanamide
83	C_5H_9N from peptides
85	$C_4H_5O_2$ from carbohydrates
91	Fragment from peptides
98/99	Carbohydrates
206, 222, 230/231, 246, 254, 258	Lignins
296, 299, 337, 418, 424	Fatty acids ($C_{19:1}$, $C_{19:0}$, $C_{22:2}$, $C_{28:3}$, $C_{28:0}$)

can be ascribed to a tillage-promoted microbial turnover of soil organic N, confirming that the hot-water extracts are a particularly sensitive approach to detect early SOM changes (Haynes, 2005).

4.2 Soil CO_2 efflux

The immediate and sharp increase of CO_2 efflux from soils just after tillage is a well-documented response and seems to be mainly driven by the release of trapped CO_2 from aggregates broken up by tillage (Reicosky et al., 1997). It is commonly suggested that a decrease in this physical outgassing a few hours afterwards is accompanied by increased soil respiration due to a better substrate supply for microorganisms from disrupted aggregates as well as increased soil aeration (Grandy and Robertson, 2007). The amounts of the observed fluxes are well in accordance with the findings of previous studies (e.g. Rochette and Angers, 1999) and can be explained by both the magnitude of the disturbance, i.e. soil comminution, and the fertilisation history of the soil (Fiedler et al., 2015).

The smaller relative efflux from BD compared to MF and CL after tillage is remarkable since before tillage the CO_2 fluxes in BD were of the same magnitude as those in MF and exceeded those in CL (Fig. 2). This becomes particularly evident when we consider the relation of cumulated CO_2 fluxes between the treatments before (19 October) and after tillage (24–29 October) (Fig. 3). The relatively lower CO_2 efflux from BD after tillage may have different reasons. On the one hand, C originating from the digestates is likely less available to soil microorganisms compared to undigested organic matter, i.e. more "recalcitrant", since the most labile C is generally consumed in the biogas reactor (Möller, 2015). On the other hand, even a single application of organic amendment can increase aggregate stability (Grandy et al., 2002). Therefore, the resilience against disruption by tillage might be promoted, leading to a better physical protection of labile soil C not contained within digestates. As a consequence, the effect of increased CO_2 efflux after tillage as observed in CL and MF may have been substantially reduced by a relative shortage of labile substrate for soil respiration in BD.

4.3 Pyrolysis-field ionisation mass spectrometry and synthesis

Generally, the Py-FIMS basic data and mass spectra (Fig. 4) and the proportions of compound classes (Table 2) confirm published data from this method for Luvisols in terms of relatively high proportions of lignin monomers, phenols and alkyl aromatics (Leinweber et al., 2009). Lignin monomers and phenols might be collectively attributed to residues of the just-harvested maize. Indeed, Gregorich et al. (1996) found that these are important components of maize leaves and roots as well as of the light fraction of the soil under this crop. Overall, the Py-FIMS data indicate differences in SOM composition between the fertilisation treatments as well as a pronounced impact of tillage in the MF and BD treatments (Fig. 5).

In the spectra of samples from BD, the additional peak at 390 °C in the TII thermogram (Fig. 4) can be attributed to mainly phenols and lignin monomers which likely originated from primary organic matter residues since this relatively low volatilisation temperature indicates labile and fairly undecomposed organic matter (Sleutel et al., 2011). It is reasonable to refer this organic matter to residues from the application of digestate. VM and TII, which are indicators of SOM content (Sorge et al., 1993), were larger in BD than in MF and CL before tillage (Table 2). This suggests a tendency to increased SOM content through application of digestate. The compound classes of BD revealed the

largest proportions of lignin dimers, lipids, sterols, suberin and free fatty acids at the expense of carbohydrates, heterocyclic N-containing compounds and peptides before tillage (Table 2). Such an SOM composition most likely reflects the cattle manure and plant residues of the biogas feedstock and their relative depletions (amides and polysaccharides) or enrichments (lignins and long-chain aliphatic compounds) during anaerobic fermentation (Möller, 2015; van Bochove et al., 1996). The pronounced tillage effect in this treatment – which is obvious from the increased relative signal intensities of carbohydrates, phenols and lignin monomers, alkyl aromatics, heterocyclic N-containing compounds and peptides at the expense of lignin dimers, lipids, sterols and free fatty acids following tillage (Table 2) – suggests the decomposition of lignin and the new formation of carbohydrates and peptides. This is in line with reports of lignin decomposition being faster than that of the total SOM (Leinweber et al., 2008; Thevenot et al., 2010). Kalbitz et al. (2003) suggested that lignin-derived moieties and lipids are utilised by microorganisms at low initial availability of carbohydrates, accompanied by an accumulation of the resulting microbial metabolites like carbohydrates and peptides. Recently, Rinkes et al. (2016) also found that decomposers may break down lignin to acquire C for their metabolism in the absence of available labile C. This suggestion is supported on the one hand by the effect of specific lignins on soil CO_2 efflux (Table 3) since CO_2 is an indicator for microbial decomposition activity (Kuzyakov, 2006). On the other hand, a relative increase in the signals for *m/z* 125, 167, 185 and 203 was observed in the BD treatment (data not shown), which are assigned to the bacterial cell wall products N-acetylmuramic acid and N-acetylmuramyl-*l*-alanyl-*d*-isoglutamine (Bahr and Schulten, 1983). Furthermore, the build-up of heterocyclic N-containing compounds might also imply a relative shortage of available carbohydrates since reduced C availability during the microbial transformation of N is suggested to promote formation of heterocyclic N instead of N immobilisation (Follett and Schimel, 1989; Gillespie et al., 2014; Schulten and Hempfling, 1992). The increased proportion of lipids at the expense of carbohydrates and peptides in MF likely results from increased heterotrophic respiration of labile substrates driven by enhanced microbial activity after tillage (La Scala et al., 2008; Zakharova et al., 2014). Decreasing proportions of carbohydrates and decreasing relative signal intensities of *m/z* 17 and 18 (data not shown), which are assigned to ammonia and ammonium, also point to a microbial immobilisation in MF (Mengel, 1996). Accordingly, these two *m/z* were also selected by the PLSR as explanatory signals for CO_2 efflux (Table 3). The minor changes in SOM compounds in CL might be a consequence of the wider HWC / HWN ratio compared to MF and BD since it indicates a lower availability of labile N for microbial utilisation (Mengel, 1996). However, the total C / N ratios were not critical for microbial activity (Table 1) (Kuzyakov et al., 2000).

A significant ($p < 0.05$) and positive correlation was observed between HWC and carbohydrates in MF. This linkage was previously described by Leinweber et al. (1995) and attributed to microbial biomass (Ghani et al., 2003) and labile soil C (Sparling et al., 1998). In contrast, this correlation was not apparent in BD. This corroborates the assumption that microorganisms in BD may have been short of available labile C. Interestingly, HWN correlated positively with carbohydrates in BD. Since the major part of carbohydrates in soils originates from microorganisms and their residues (Gunina and Kuzyakov, 2015), this may suggest a metabolic coupling between carbohydrates and HWN because many N-cycling processes are mediated microbially (Isobe and Ohte, 2014).

Increased amounts of sterols are typically found in biogas digestates (Leinweber, 2016, unpublished Py-FIMS data). In BD, the cumulated CO_2 efflux and the amount of sterols was negatively correlated. This supports the suggestion of Heumann et al. (2011, 2013) that sterols may have an inhibitory effect on microorganisms of the N cycle and thus may slow down soil respiration. However, since the amounts of sterols decreased significantly after tillage in BD (Table 3), the actual sterol contribution to reduced CO_2 efflux in BD relative to the other treatments cannot be ascertained by the present data set.

Our data and analyses suggest a short-term induction of enhanced microbial N-turnover by tillage in soils amended with biogas digestates, possibly co-occurring with the decomposition of lignin as a C source due to a relative shortage of carbohydrates. This is supported by the results of each of the methods used, i.e. (i) HWN as an indicator for labile N increased; (ii) lignins, ammonia and ammonium discriminated as explanatory variables for cumulated CO_2 efflux by PLSR; and (iii) Py-FIMS data point at an increase in N-containing compounds along with decomposition of lignins and formation of carbohydrates and peptides.

In MF, the depletion of HWC was linked to decreasing amounts of carbohydrates, certainly due to increased microbial respiration, though no significant correlation with CO_2 efflux was found. No modifications were detected in CL, where the absence of amendment may have led to a relative shortage of labile N as indicated by the higher HWC / HWN ratio, which possibly prevented an enhanced microbial activity.

4.4 Limitations

Although the relatively small sampling areas around the bases in each treatment plot might suggest a "pseudoreplication" in soil sampling, we have evidence suggesting very high spatial variability in the soil, which alleviates this problem: in a master's thesis on spatial variability, Jacobs (2014) revealed that N_2O fluxes from the soil of the study site show very high small-scale variability well below the metre scale. Therefore, we assume "real" (i.e. independent) replicates, though the comparison *between* the treat-

ments should be done carefully because of possibly rather small differences. Thus, due to the potentially lowered influence of spatial variability, our sampling design might have biased our results towards the detection of even small temporal changes *within* the treatments. Because we are mainly interested in the impact of tillage, this limitation does not interfere with our findings.

5 Conclusions

Combining Py-FIMS, as a sensitive technique to detect differences and alterations of specific compound classes of SOM, with classical methods like hot-water extraction and measurements of soil CO_2 efflux allowed us to gain a better understanding of short-term SOM turnover after tillage operations. After tillage, SOM composition of the investigated soil changed in the temporal scale of days and the changes varied significantly under different types of amendment. Particularly obvious were the turnover of lignin-derived substances and the depletion of carbohydrates due to soil respiration. Thus, in BD, the SOM turnover was relatively fast, questioning the suggested recalcitrance of biogas digestates as stable leftovers of the anaerobic fermentation. Since we found indications of inhibitory effects of sterols on the CO_2 efflux, which were previously reported in three independent studies on parameters of the N cycle, their long-term impact on SOM stocks should be examined more closely. Therefore, future investigations should address the short- and long-term turnover of SOM following various amendments, especially with the relatively new biogas digestates.

6 Data availability

The underlying research data can be accessed publicly via the Supplement.

The Supplement related to this article is available online at doi:10.5194/soil-2-475-2016-supplement.

Acknowledgements. We thank the technicians Steffen Kaufmane and Sascha Tittmar for their assistance during field work and the research facility for agriculture and fisheries of the federal state of Mecklenburg-Western Pomerania (LFA) in Gülzow for their co-operation, especially Jana Peters and Andreas Gurgel. We also thank Karsten Kalbitz, Steven Sleutel and an anonymous referee for their constructive criticism and suggestions regarding the manuscript. The joint research project underlying this report was funded by the German Federal Ministry of Food and Agriculture (funding identifier 22007910). Py-FIMS analyses in the Mass Spectrometry Laboratory of Soil Science were funded by the "Exzellenzförderprogramm" of the Ministry of Education, Science and Culture, federal state of Mecklenburg-Western Pomerania (project UR 07 079) as well as by the German Federal Ministry of Food and Agriculture (funding identifier 22400112).

Edited by: K. Kalbitz
Reviewed by: S. Sleutel and one anonymous referee

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4 Potential short-term losses of N_2O and N_2 from high concentrations of biogas digestate in arable soils

SOIL, 3, 161–176, 2017
https://doi.org/10.5194/soil-3-161-2017

SOIL Open Access EGU

Potential short-term losses of N_2O and N_2 from high concentrations of biogas digestate in arable soils

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Received: 9 February 2017 – Discussion started: 28 February 2017
Revised: 14 August 2017 – Accepted: 27 August 2017 – Published: 28 September 2017

Abstract. Biogas digestate (BD) is increasingly used as organic fertilizer, but has a high potential for NH_3 losses. Its proposed injection into soils as a countermeasure has been suggested to promote the generation of N_2O, leading to a potential trade-off. Furthermore, the effect of high nutrient concentrations on N_2 losses as they may appear after injection of BD into soil has not yet been evaluated. Hence, we performed an incubation experiment with soil cores in a helium–oxygen atmosphere to examine the influence of soil substrate (loamy sand, clayey silt), water-filled pore space (WFPS; 35, 55, 75 %) and application rate (0, 17.6 and 35.2 mL BD per soil core, 250 cm^3) on the emission of N_2O, N_2 and CO_2 after the usage of high loads of BD. To determine the potential capacity for gaseous losses, we applied anaerobic conditions by purging with helium for the last 24 h of incubation. Immediate N_2O and N_2 emissions as well as the N_2 / (N_2O+N_2) product ratio depended on soil type and increased with WFPS, indicating a crucial role of soil gas diffusivity for the formation and emission of nitrogenous gases in agricultural soils. However, emissions did not increase with the application rate of BD. This is probably due to an inhibitory effect of the high NH_4^+ content of BD on nitrification. Our results suggest a larger potential for N_2O formation immediately following BD injection in the fine-textured clayey silt compared to the coarse loamy sand. By contrast, the loamy sand showed a higher potential for N_2 production under anaerobic conditions. Our results suggest that short-term N losses of N_2O and N_2 after injection may be higher than probable losses of NH_3 following surface application of BD.

1 Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas (Myhre et al., 2013), with agriculture being its single largest anthropogenic source, contributing about 4.1 Tg N_2O-N yr^{-1} or 66 % of total gross anthropogenic emissions, mainly as a result of mineral nitrogen (N) fertilizer and manure application (Davidson and Kanter, 2014). The generation of nitrogen gas (N_2) is of agronomic interest in terms of nutrient management since such gaseous losses may imply a significant loss of N from the soil–plant system (Cameron et al., 2013; Friedl et al., 2016). However, from an environmental stance, N_2 is innocuous and, thus, the preferred type of gaseous N loss from soil (Davidson et al., 2015). In general, the improvement of N use efficiency and thus the decrease in N losses in crop production are paramount in the presence of challenges like food security, environmental degradation and climate change (Zhang et al., 2015).

Digestion residues (biogas digestate, BD) from biogas plants are used as organic amendment in agriculture. However, compared to undigested amendments, digestion results in an increased pH, a higher proportion of ammonium (NH_4^+) and a narrowed C / N ratio of BD (Möller and Müller, 2012). These altered chemical properties may promote biochemical

reactions in the soil that are responsible for the formation of gaseous N species like N_2O, nitric oxide (NO), N_2 and ammonia (NH_3) (Nkoa, 2013).

Significant losses of N as NH_3 may occur within the first hours after manure application (Quakernack et al., 2012). To reduce NH_3 losses, the application of BD by injection is recommended, but this measure can simultaneously increase the potential for N_2O losses compared to surface application (Velthof and Mosquera, 2011; Wulf et al., 2002). On the one hand, high NH_4^+ concentrations in the injection band promote nitrification, consuming O_2 and releasing N_2O (Christensen and Rowe, 1984). On the other hand, increased amounts of C in the injection band also promote respiration, additionally depleting O_2 supply (Dell et al., 2011). Altogether, the conditions during the initial phase after injection of BD foster microsites favourable for microbial denitrification, which also promote the formation of N_2 due to anaerobic conditions (Köster et al., 2015; Webb et al., 2010).

There is a wealth of biotic and abiotic processes in soils that produce N_2O and N_2, depending on mineral N content, C availability and temperature, most of which are enhanced by anoxic or at least suboxic conditions (Butterbach-Bahl et al., 2013). The amounts and the relative share of N_2 and N_2O in the overall gaseous N emissions depend – among other factors – on the degree of O_2 restriction (Firestone and Davidson, 1989). Soil physical and biotic factors (i.e. diffusion permitted by soil porosity in conjunction with water-filled pore space (WFPS) as well as consumption of O_2 by heterotrophic respiration and nitrification) control the aerobic status of a soil (Ball, 2013; Maag and Vinther, 1999; Uchida et al., 2008). In general, clayey soils exhibit a lower gas diffusivity compared to coarse-textured soils. This regularly results in higher denitrification in the former with higher N_2O emission rates, but also a higher probability for the consecutive reduction to N_2 (Ball, 2013; Gu et al., 2013; Senbayram et al., 2014).

There is a general lack of knowledge about the effects of high BD concentration on gaseous N losses as they might appear after injection into soils and their interactions with O_2 limiting factors like soil texture and WFPS, as well as temperature and heterotrophic respiration. Thus, we applied the helium–oxygen (He–O_2) incubation technique (Butterbach-Bahl et al., 2002) in a laboratory experiment to evaluate the effect of the factors suggested above on the emission of N_2O and N_2 from different soils. Simultaneously, CO_2 flux was determined as an indicator for microbial O_2 consumption, O_2 diffusion and also for the degradability of organic C applied with BD (Blagodatsky and Smith, 2012). We hypothesized that (1) N_2O and N_2 emissions will increase with WFPS, (2) gaseous N losses will also be affected by BD application rate, i.e. the hypothetical concentration of C and N resulting from injection, and (3) the clayey silt will induce higher gaseous N losses than the coarse loamy sand.

Table 1. The examined factors soil texture, water-filled pore space (WFPS) and amount (i.e. concentration) of nitrogen (N) applied with biogas digestate (BD) with their respective levels applied in the present study, resulting in 18 treatments with three replicates each. The temperature was manipulated consecutively during the incubation.

Factor (n)	Levels		
Soil texture (2)	loamy sand	clayey silt	
WFPS (%) (3)	35	55	75
BD-N (kg ha^{-1}) (3)	0	160	320
Temperature (°C) (2)	2	15	

2 Material and methods

2.1 Selected soils, sampling of soil cores and biogas digestate

Two soils were selected and both were adjusted to three levels of WFPS and three quantities of BD (Table 1), resulting in 18 factor combinations with three replicates. Temperature was increased from 2 °C during the first 2 days to 15 °C for the last 3 days of the incubation. Intact soil cores (diameter 7.2 cm, height 6.1 cm, volume 250 cm^3) were taken with sample rings in the range from 0 to 0.10 m depth from two sites with different textures, i.e. sandy loam and clayey silt. The sandy loam samples were collected from a Stagnic Luvisol (IUSS Working Group WRB, 2006) located in Gülzow (northeast Germany) in the ground moraine of the Weichselian glacial period at 53°48′35″ N and 12°4′20″ E. The clayey silt samples were collected from a Haplic Luvisol located in Dornburg between the foothills and the lowlands of central Germany at 51°0′8″ N and 11°39′25″ E (see Table 2 for more details on soil characteristics). After field sampling, the soil cores were dried for 48 h at 40 °C to facilitate adjustment of WFPS.

Both sites have been cultivated with similar crop rotations used as feedstock for biogas production and have been amended with BD for the past 9 years. The crop rotation on the sandy loam consisted of maize (*Zea mays* L.), rye (*Secale cereale* L.), sorghum (*Sorghum bicolor* (L.) Moench), winter triticale (× *Triticosecale* Wittmack), ryegrass (*Lolium perenne* L.) and winter wheat (*Triticum aestivum* L.). The only difference in the crop rotation on the clayey silt was the cultivation of sudan grass (*Sorghum* × *drummondii*) instead of sorghum.

The BD used for the incubation was obtained from a biogas plant at "Gut Dalwitz", an organic farm in northeast Germany. The feedstock for the anaerobic fermentation in the plant consisted of 60 % maize, 20 % solid cattle manure, 10 % dry chicken manure and 10 % rye. The digestate was analysed by LUFA Rostock, Germany, and had a pH of 8.3, 2.91 % organic C, 0.16 % dissolved organic C (DOC), 0.54 %

Table 2. Characteristics of both soils. Texture and mean values with standard deviations (in parentheses) for carbon (C, $n = 9$), nitrogen (N, $n = 9$), pH ($n = 3$), bulk density (BD, $n = 3$) and mineral N (NO_3^- and NH_4^+, $n = 3$) of both soils in 0–10 cm depth after field sampling.

Texture	C ($mg\,g^{-1}$)[a]	N ($mg\,g^{-1}$)[a]	pH[b]	Bulk density ($g\,cm^{-3}$)[c]	NO_3^- ($mg\,kg^{-1}$)[d]	NH_4^+ ($mg\,kg^{-1}$)[d]
Loamy sand	6.99 (0.29)	0.67 (0.05)	7.2 (0.1)	1.4 (0.0)	1.0 (0.2)	0.6 (0.3)
Clayey silt	10.77 (0.28)	1.19 (0.06)	7.2 (0.0)	1.5 (0.0)	1.8 (0.2)	0.3 (0.2)

[a] Measured with the analyser TruSpec CNS, Leco Instruments GmbH, Germany; performed according to ISO 10694 (elemental analysis) for C and according to ISO 13878 (elemental analysis) for N. [b] Measured in H_2O with TitraMaster 85, Radiometer Analytical SAS, France; performed according to *VDLUFA Methodenbuch*, Vol. 1, chap. 5.1.1. [c] Measured on 250 cm^3 soil cores. [d] Measured with the analyser CFA-SAN, Skalar Analytical B.V., the Netherlands; performed according to ISO 14256.

Table 3. Chronological order of the incubated factor combinations. Two different factor combinations with their respective repetitions ($n = 3$) were placed together for each weekly incubation course (see Fig. 1). The factors were combined by (1) soil (loamy sand: LS, clayey silt: CS), (2) amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD) and (3) WFPS (%).

Week	Factor combination 1	Factor combination 2
1	LS – No BD – 35 %	LS – No BD – 55 %
2	LS – No BD – 75 %	LS – Low BD – 35 %
3	LS – Low BD – 55 %	LS – Low BD – 75 %
4	LS – High BD – 35 %	LS – High BD – 55 %
5	LS – High BD – 75 %	CS – No BD – 35 %
6	CS – No BD – 55 %	CS – No BD – 75 %
7	CS – Low BD – 35 %	CS – Low BD – 55 %
8	CS – Low BD – 75 %	CS – High BD – 35 %
9	CS – High BD – 55 %	CS – High BD – 75 %

N and 0.27 % NH_4-N in undried material with a dry matter content of 9.4 %.

2.2 Adjustment of WFPS and addition of N

For adjustment of WFPS, the dry and undisturbed soil cores were moistened dropwise. The respective quantities of water were calculated based on the bulk density and an assumed particle density of 2.65 $g\,cm^{-1}$ and they were reduced by the expected moisture input from subsequent addition of BD. The soil cores were then mixed with BD and finally repacked to reach nutrient concentrations comparable to those in injection bands. The amounts of added BD were calculated with an assumed injection of 160 kg N ha^{-1} into the soil, with row spaces of 0.15 m (narrow injection bands with low BD concentration, LOBD) and 0.30 m (wide injection bands with high BD concentration, HIBD). These are common ranges used for injection machinery and correspond to 17.6 and 25.3 mL BD per sample ring. After this procedure, the soil cores were sealed with plastic lids and stored immediately at 2 °C until the beginning of the incubation within a week.

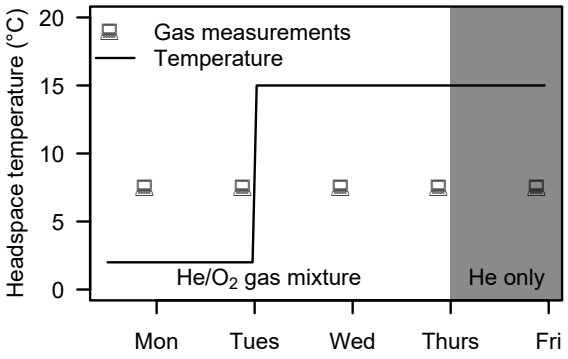

Figure 1. Course of incubation and gas measurements with respect to atmosphere and temperature of the headspace after 2 days of pre-incubation at 2 °C in the He / O_2 gas mixture. Gas concentrations of the headspace were determined on 5 consecutive days, i.e. Monday to Friday in the morning. After the first 2 measurement days, the headspace temperature was increased from 2 to 15 °C. Additionally, after the fourth measurement day, the aerobic helium / oxygen gas mixture in the headspace was replaced by a pure helium atmosphere.

2.3 Determination of gas fluxes

The measurements of N_2, N_2O and CO_2 fluxes were applied following the He–O_2 method (Butterbach-Bahl et al., 2002; Scholefield et al., 1997). Six soil cores (i.e. the repetitions of two factor combinations at a time, Table 3) were placed simultaneously in special gas-tight incubation vessels inside a climate chamber. Analyses were conducted in the laboratory of the Institute for Landscape Biogeochemistry, Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany. Before flux measurements, the vessels were evacuated to 0.047 bar and flushed with an artificial He / O_2 gas mixture (20.49 % O_2, 345.5 ppm CO_2, 359 ppb N_2O, 1863 ppb CH_4, 2.46 ppm N_2, rest He) four times consecutively to remove ambient N_2. Subsequently, the air temperature of the climate chamber was set to 2 °C and a continuous He / O_2 gas flow rate of 15 mL min^{-1} was applied to the vessel headspace for 72 h to remove residues of N_2 from soil cores by diffusion, including a restricted N_2 production by decreased microbial activity. After this pre-incubation, on the following 2 days the headspace concentration of N_2O and

CO_2 was measured once daily in the morning. To compensate for the lower precision of the detector for N_2 in relation to the detector for N_2O and CO_2 (Eickenscheidt et al., 2014), N_2 concentrations were measured consecutively three times daily in the morning. Immediately after the last measurement on the second day, the temperature was set to 15 °C and the measurements were continued for another 2 days. Finally, the He / O_2 gas mixture was substituted by pure He and, following 24 h of acclimatization, gas measurements were carried out once again (Fig. 1) to determine the generation of N_2O and N_2 in a completely anaerobic soil matrix. The latter step is important to get a clue about the actual potential for gaseous N losses after highly concentrated BD application. The settings of the chromatographs for gas analyses are described in Eickenscheidt et al. (2014). Gas fluxes were calculated according to Eq. (1):

$$f = \frac{M \times p \times v \times dc}{R \times T \times A}, \quad (1)$$

where f is the flux (N_2 and CO_2: mg m^{-2} h^{-1}, N_2O: µg m^{-2} h^{-1}), M the molar mass in g mol^{-1} (N_2: 28, CO_2: 44, N_2O: 44), p the air pressure (Pa), v the air flow (L h^{-1}), R the gas constant (8.31 J mol^{-1} K^{-1}), T the temperature inside the chamber (K), A the area of the incubation vessel (m^2) and dc the difference of gas concentrations (N_2 and CO_2: ppm, N_2O: ppb) between inlet and outlet of a vessel.

To enhance the tightness against atmospheric N_2 contamination, the lids of the incubation vessels were permanently purged with helium. We obtained blank values by inserting aluminium blocks into the vessels before each measurement cycle. Since these blank values were usually steady with means of 1.9 ($1\sigma = 0.9$) ppm N_2, 349.6 ($1\sigma = 11.4$) ppb N_2O and 353.9 ($1\sigma = 13.5$) ppm CO_2, we suggest that the vessels were tight. Derived from the blank values, the lowest detectable fluxes were on average 0.427 ($1\sigma = 0.271$) mg N_2-N m^{-2} h^{-1}, 3.6 (3.1) µg N_2O-N m^{-2} h^{-1} and 0.918 (0.693) mg CO_2-C m^{-2} h^{-1}. For flux estimation, the blank values were subtracted from the values measured at the respective outlet. Estimated fluxes from the soil cores smaller than the respective blank fluxes of each day were set to zero.

2.4 Soil analyses after incubation

After incubation, the soil cores were stored at 2 °C until they were extracted with 0.1 M KCl solution (the soil-to-extract ratio was 1 : 4; standardized extraction method of the commissioned laboratory at Leibniz Centre for Agricultural Landscape Research e.V.) and analysed for NH_4^+ and nitrate (NO_3^-) using spectrophotometry according to DIN ISO 14256 with the continuous flow analyser CFA-SAN, Skalar Analytical B.V., the Netherlands, and for DOC by combustion according to DIN ISO 10694 with the analyser RC 612, Leco Instruments GmbH, Germany.

2.5 Statistical analysis

All statistical analyses were done using R statistical software version 3.2.3 (R Core Team, 2016) with the data of the measuring days under a He–O_2 atmosphere. Data from the vessels with the factor combination of 35 % WFPS and LOBD with clayey silt were omitted due to technical reasons during sample preparation. For the final period of pure He headspace, some gas concentration data could not be documented. For loamy sand, this affects all WFPS levels with LOBD (N_2 and N_2O), the treatment 75 % WFPS with 320 kg N h^{-1} (N_2O and CO_2) and for the clayey silt the treatment 35 % WFPS without amendment (N_2O and CO_2).

To account for repeated measurement of vessels, linear mixed effect models were applied with the R package "lmerTest" version 2.0-33 (Kuznetsova et al., 2016) for fluxes of each gas type. The three pseudo-replicated fluxes from the N_2 measurements of each vessel were averaged for each day to obtain the same number of observations as for N_2O and CO_2 fluxes. The fixed structure of models included soil type, WFPS, amount of digestate, temperature, NO_3^- and DOC contents after incubation as well as the fluxes of N_2O (in the model for N_2) and CO_2 (in the models for N_2, N_2O and N_2 / [N_2+N_2O] product ratio). Soil NH_4^+ was omitted since it showed high autocorrelation with the amount of BD applied. The individual soil cores in the vessels were set as random effect (nested within the week of incubation and with allowance for a variable slope of the effect each day) with regard to lack of independence of consecutive measurements. The model responses for N_2, N_2O and CO_2 were log transformed (ln[value + 1]) since gas fluxes from soils usually show lognormal distributions (Kaiser et al., 1998). The function "step" was used for automatic backward selection of models based on AIC (Akaike's information criterion). The skewness (γ) was calculated with the R package "moments" version 0.14 (Komsta and Novomestky, 2015) to check residuals for normal distribution and $|\gamma| \leq 2$ was assumed as appropriate (West et al., 1995). For mixed-effect models, p values of the ANOVA (type 2) were calculated based on Satterthwaite's approximation.

Cumulated gas fluxes were estimated with a bootstrap method using function "auc.mc" of the R package "flux" version 0.3-0 (Jurasinski et al., 2014). In short, the fluxes for the period of aerobic headspace were cumulated in 100 iterations, while for each run two fluxes were omitted randomly. Then, the resulting data were used to calculate means and standard deviations.

3 Results

3.1 Soil NH_4^+, NO_3^- and DOC contents

The calculated application of NH_4^+-N from BD per kilogram of soil approximated for the sandy loam was 135.8 mg (LOBD) and 271.5 mg (HIBD), and for the clayey silt it was

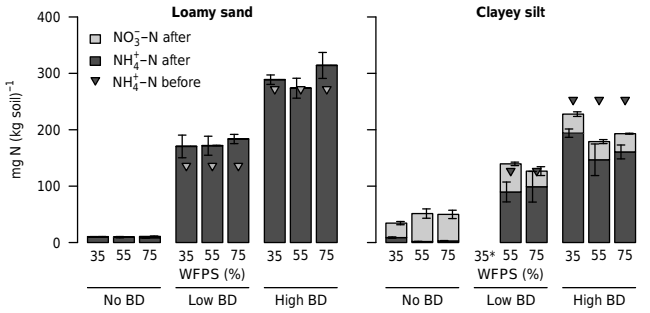

Figure 2. Ammonium and nitrate contents from loamy sand and clayey silt after incubation with different water-filled pore spaces (WFPSs, %) and amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD). Error bars denote standard deviations. In general, the ammonium content increased with digestate application, with lower amounts detected in the clayey silt. Nitrate was found almost exclusively in the latter soil. For comparison, inverted triangles show calculated amounts of applied ammonium, which may differ from actual rates due to heterogeneity of biogas digestate. One treatment (*) was omitted from all analyses due to technical reasons.

126.7 mg (LOBD) and 253.4 mg (HIBD). The NO_3^- content of BD was negligible. In general, the NH_4^+ content of the soils after incubation increased with digestate application, with lower amounts detected in the clayey silt. Nitrate was found almost exclusively in the latter soil (Fig. 2).

The amounts of measured DOC increased with the application rate of BD, but with higher magnitudes for the loamy sand than for the clayey silt (Table 4).

3.2 CO_2 fluxes

CO_2 fluxes showed clear differences between the soils: under all combinations of temperature and oxygen, the fluxes were always larger from loamy sand compared to clayey silt (Table A1). In general, mean fluxes from loamy sand increased with the amount of digestate during each of the different periods regarding temperature and headspace aerobicity, but showed no obvious pattern with WFPS. There was no clear trend of fluxes with the amount of amendment, but a slight trend of decreasing fluxes with increasing WFPS could be seen for the clayey silt. However, the predictive power of WFPS on CO_2-C fluxes was minor since it was eliminated during stepwise regression fitting. By contrast, soil type, amount of digestate, temperature and the DOC content after the incubation had significant ($p < 0.01$) effects (Table 5).

3.3 N_2O fluxes

The mean N_2O fluxes from the loamy sand in the He–O_2 headspace were virtually zero, independent of temperature and WFPS as well as the amount of BD application (Fig. 3, Table A2). In contrast, the emissions of the clayey silt increased with temperature and were highest at 15 °C with intermediate WFPS and amount of BD, i.e. 6.2 mg N_2O-N m^{-2} h^{-1} at 55 % with LOBD, respectively. Surprisingly, at 15 °C, increasing the amount of BD up to HIBD did not increase the observed N_2O efflux; rather, it decreased the efflux significantly ($p < 0.05$, Tukey's HSD) at 55 % and also, but not significantly, at 75 % WFPS (Fig. 3, Table A2). According to the linear mixed model for N_2O fluxes in aerobic conditions, WFPS, amount of digestate, temperature, DOC content of soil after incubation and CO_2 fluxes had significant ($p < 0.001$) effects on N_2O flux (Table 5).

Under anaerobic headspace conditions, the overall highest mean N_2O flux was observed from the clayey silt at 35 % WFPS with HIBD (11.7 mg N_2O-N m^{-2} h^{-1}). The same soil showed a tendency of decreasing N_2O fluxes with increasing WFPS and amendment. In the loamy sand, the pure He atmosphere induced increasing mean N_2O fluxes (up to 1.3 mg N_2O-N m^{-2} h^{-1}) with increasing WFPS (Fig. 3, Table A2). Thus, the anaerobic headspace only induced a change in the loamy sand by increasing emissions.

3.4 N_2 fluxes

From the loamy sand, no or only small rates of N_2 were detected at both temperatures under a He–O_2 atmosphere (Fig. 4, Table A3). The clayey silt showed mean fluxes of up to 1.4 mg N_2 m^{-2} h^{-1} at 2 °C (all incubations with 75 % WFPS) and up to 3.8 mg N_2 m^{-2} h^{-1} at 15 °C (75 % WFPS with LOBD), but no fluxes in all BD treatments with 35 % WFPS. Put simply, temperature had a small effect on N_2 emissions from the sandy loam, with no consistent influence of WFPS and the amount of BD. In contrast, the clayey silt emitted increasing fluxes with increasing temperature and WFPS. However, the application rise from LOBD up to HIBD at 15 °C resulted in slightly, but not significantly ($p > 0.05$, Tukey's HSD) decreased fluxes (Fig. 4, Table A3). The summary of the linear mixed model for N_2 fluxes under aerobic conditions revealed significant effects ($p < 0.05$) of soil type, WFPS, the amount of digestate, temperature, DOC content after incubation and N_2O flux (Table 5).

After switching the atmosphere to pure He, the N_2 fluxes from the sandy loam increased more than 60-fold. In contrast to aerobic conditions, all measured factor combinations showed mean fluxes of up to 35.1 mg N_2 m^{-2} h^{-1} (55 % with 320 kg N ha^{-1}) (Fig. 2, day 5 in Table A3). Mean fluxes from clayey silt increased only up to 9.3 mg N_2 m^{-2} h^{-1} in amended treatments. Thus, the loamy sand exhibited a much more intense reaction under anaerobic headspace conditions.

3.5 N_2 / (N_2 + N_2O-N) product ratio

No clear trend of the product ratio of N_2 / (N_2 + N_2O-N) was found for incubations of the loamy sand. However, there was a clear distinction between the ratios for this soil under aero-

Table 4. Mean DOC values from soils, measured after incubation, with standard deviations in parentheses for the respective treatments differing in amount of applied biogas digestate (BD) and water-filled pore space (WFPS).

kg digestate-N ha^{-1}	WFPS (%)	mg DOC (kg soil)$^{-1}$	
		Loamy sand	Clayey silt
0	35	41.4 (2.7)	18.9 (1.1)
	55	38.6 (3.1)	19.8 (1.4)
	75	43.7 (1.4)	19.0 (1.8)
160	35	197.4 (20.7)	NA
	55	190.5 (19.3)	68.3 (12.7)
	75	362.2 (40.0)	63.2 (9.6)
320	35	316.8 (25.3)	358.1 (26.3)
	55	312.5 (14.3)	94.8 (13.6)
	75	500.1 (33.4)	105.9 (14.8)

NA: data not available.

Table 5. ANOVA table (type 2, p values calculated based on Satterthwaite's approximation) of the linear mixed-effect models for estimated fluxes of N_2, N_2O, N_2 / (N_2+N_2O) product ratio and CO_2 in an aerobic He–O_2 atmosphere. Soil type, water-filled pore space (WFPS), amount of digestate, temperature, NO_3^- and DOC content of soil after incubation, and fluxes of N_2O and CO_2 were set as possible independent variables. The individual soil rings were set as the random effect (nested within the respective week and with the allowance for varying slopes for each day of measurements). The random effect was always significant.

Response	Fixed effects							
	Soil type	WFPS	Digestate amount	Temperature	NO_3^- post	DOC post	N_2O flux	CO_2 flux
N_2	0.026	< 0.001	0.008	0.037	[a]	0.001	< 0.001	[a]
N_2O	[a]	< 0.001	< 0.001	< 0.001	[a]	< 0.001	[b]	< 0.001
N_2 / (N_2+N_2O)	0.005	0.004	[a]	[a]	[a]	[a]	[b]	[a]
CO_2	< 0.001	[a]	< 0.001	< 0.001	[a]	0.007	[a]	[b]

[a] Variable eliminated during stepwise regression selection. [b] Variable was not included in the original regression.

bic and anaerobic atmospheres: while the ratios were close to zero in the former, they were close to 1 in the latter (Fig. 5). In contrast, in the clayey silt the ratios increased with WFPS and were affected by digestate amendment under both the aerobic and the anaerobic atmospheres, in which the highest ratios (up to 0.8) were found in treatments without digestate and at least 55 % WFPS. The digestate-amended treatments showed ratios around or above 0.5, with the exception of the 35 % WFPS treatments, which had ratios close to zero. According to the linear mixed model, the product ratio under aerobic conditions was affected significantly ($p < 0.01$) by soil type and the amount of digestate (Table 5).

4 Discussion

4.1 Increased BD application rate did not increase N_2O and N_2 losses, probably due to inhibitory effect of high NH_4^+ concentrations

In the loamy sand, the higher NH_4^+ content measured after the incubation cycle compared to the calculated NH_4^+ application rates may result from heterogeneity of BD itself (Andruschkewitsch et al., 2013). By contrast, the considerable lower values after incubation in the clayey silt could be attributed to a higher fixation of NH_4^+ as NH_3 by clay minerals, enhanced by the increased pH of BD (Kissel et al., 2008).

The overall N_2O fluxes corresponded well with those from other studies with similar incubation conditions and application rates of BD in terms of nitrogen per hectare (Köster et al., 2015; Senbayram et al., 2012; Severin et al., 2015). However, the latter studies assumed a distribution of BD into soil using a cultivator, which implies a smaller concentration of BD than we actually applied. Although we observed differences in N_2O emissions between soils, soil type was not confirmed as a significant effect. Nevertheless, WFPS and temperature, which are well-known controls of N_2O generation (Maag and Vinther, 1999), showed significant influences. Both are physical (by gas diffusion) and biological (by increased metabolic activity and consequently increased O_2 consumption by respiration) drivers for O_2 availability (Ball, 2013; Maag and Vinther, 1999). Accordingly, CO_2 flux (resulting from respiration of O_2) generally increased with tem-

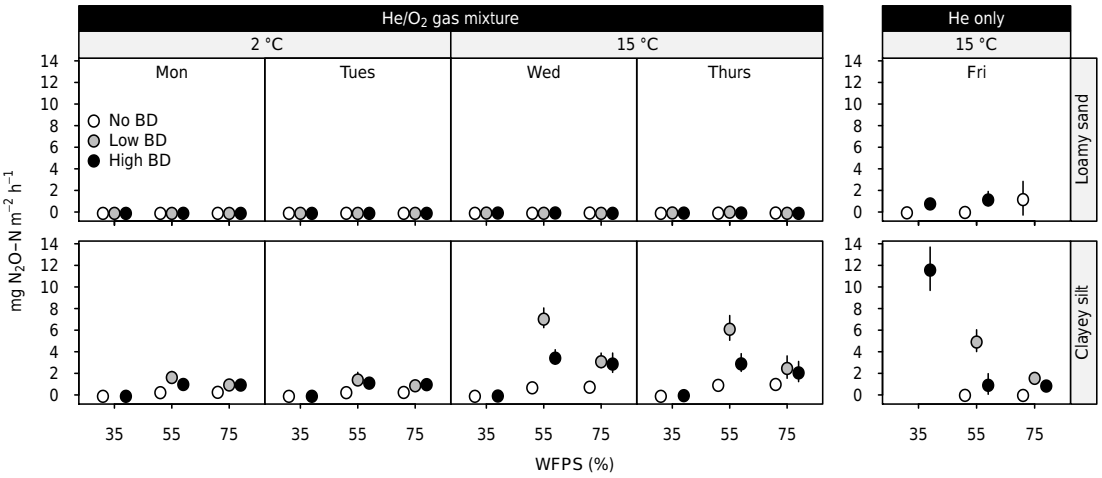

Figure 3. Mean N_2O fluxes (mg N m^{-2} h^{-1}) from loamy sand and clayey silt incubated under different water-filled pore spaces (WFPSs, %) with different amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD). The first to the fourth days of the incubation were measured in an aerobic He–O_2 headspace (with 2 days at 2 °C followed by another 2 days at 15 °C) while on the fifth day measurements were conducted in an anaerobic headspace with pure He (at 15 °C). Error bars show standard deviations; if bars are not visible, they are smaller than the symbols of the means. Under an aerobic atmosphere, N_2O fluxes from loamy sand were negligible, while fluxes from clayey silt showed an increase with temperature, especially with higher WFPS and intermediate amounts of digestate. Under an anaerobic atmosphere, mean fluxes from loamy sand increased slightly, but significantly (Tukey's HSD, $p < 0.05$). The fluxes from clayey silt showed no significant differences (Tukey's HSD, $p < 0.05$) compared to the day before, with the exception of 35 % WFPS, at which mean flux increased strongly in the treatment with 320 kg digestate N ha^{-1}.

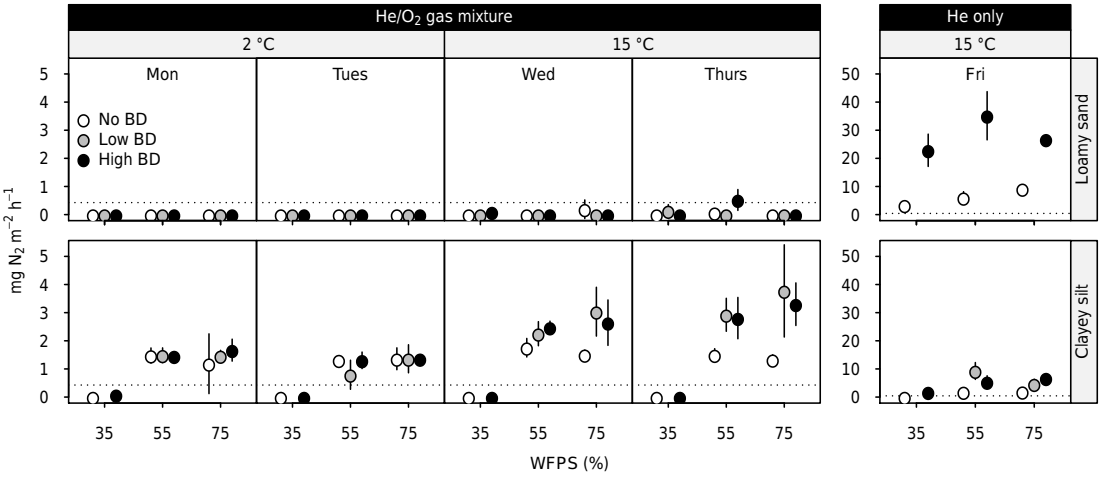

Figure 4. Mean N_2 fluxes (mg m^{-2} h^{-1}) from loamy sand and clayey silt incubated under different water-filled pore spaces (WFPSs, %) with different amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD). The first to the fourth days of the incubation were measured in an aerobic He–O_2 headspace (with 2 days at 2 °C followed by another 2 days at 15 °C) while on the fifth day measurements were conducted in an anaerobic headspace with pure He (at 15 °C). Error bars show standard deviations; if bars are not visible, they are smaller than the symbols of the means. The dotted horizontal lines depict the average blank value; single flux rates lower than the respective lank value were set zero. Under an aerobic atmosphere, N_2 fluxes from loamy sand were zero or rather negligible, while fluxes from clayey silt show a distinct increase with WFPS and higher fluxes at 15 °C. Under an anaerobic atmosphere, mean fluxes from loamy sand increased by orders of magnitude, while the fluxes from clayey silt increased as well, but more gently compared to the sand.

perature and was also identified as significant by regression selection.

The mean N_2 fluxes of up to 0.5 (loamy sand) and 3.8 mg N m^{-2} h^{-1} (clayey silt) at 15 °C (Fig. 5, Table A3) were considerably smaller than the mean fluxes of up to 13.0 mg m^{-2} h^{-1} observed by Köster et al. (2015) during the first 5 days of their incubation. Although the amount of BD in terms of applied N (250 kg ha^{-1}) was comparable, Köster

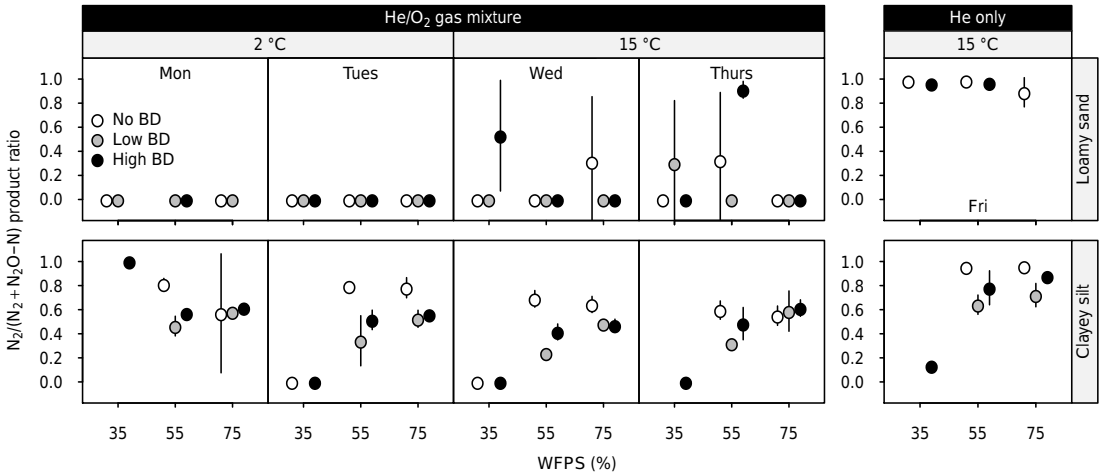

Figure 5. Mean N_2 / (N_2+ N_2O-N) product ratio from loamy sand and clayey silt incubated under different water-filled pore spaces (WFPSs, %) with different amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD). The first to the fourth days of the incubation were measured in an aerobic He–O_2 headspace (with 2 days at 2 °C followed by another 2 days at 15 °C) while on the fifth day measurements were conducted in an anaerobic headspace with pure He (at 15 °C). Error bars show standard deviations; if bars are not visible, they are smaller than the symbols of the means. For the loamy sand, there was a clear distinction of the ratios between aerobic and anaerobic atmospheres: while the ratios tended to 0 in the former, they tended to 1 in the latter, regardless of temperature or amount of digestate. For the clayey silt, ratios increased with WFPS and were highest from the unamended treatments under both the aerobic and the anaerobic atmospheres.

et al. (2015) used a higher WFPS of 90 %, which may have increased the generation of N_2. In contrast to N_2O emission rates, the observed N_2 fluxes depended not only on WFPS but also on soil type (Table 5). This is most likely due to the direct influence of soil structure on diffusivity and the resulting supply with O_2 (Balaine et al., 2016; Butterbach-Bahl et al., 2013). N_2O flux also showed a significant effect during regression selection for N_2. N_2O is the direct precursor of N_2 in denitrification; thus, the flux of the latter depends on the availability of the former. However, temperature showed no significant effect.

N_2 / (N_2+ N_2O) product ratios were significantly determined only by soil type and WFPS: while no clear trend was observable for the loamy sand, there was a pronounced effect in the clayey silt (Fig. 4). We attribute the lack of a trend in loamy sand to generally adverse conditions for the formation of N_2O and N_2, i.e. a sufficient supply of O_2 (see Sect. 4.2). Conversely, the influence of WFPS apparently mirrored favourable conditions in the clayey silt (Table 5). Simultaneously, with increasing WFPS, the reduction of N_2O as an alternative electron acceptor under reduced O_2 supply accelerates (Tiedje, 1988). Accordingly, no or rather small fluxes of the investigated gaseous N species were generally found in our presumably well-aerated treatments with 35 % WFPS.

In our study, one treatment (clayey silt, 55 % WFPS, LOBD) showed exceptionally large mean N_2O fluxes of up to 7.1 mg N m^{-2} h^{-1} (Fig. 3, Table A2). This could be evidence that the injection of such commonly applied amounts of BD-N (i.e. 160 kg N ha^{-1}) may favour much larger losses of N_2O compared to an even distribution of BD in a soil surface due to larger substrate concentration in injection slits. However, with higher amendments (i.e. HIBD), we observed partially significant ($p < 0.05$, Tukey's HSD) reductions of N_2O and a decreasing tendency of N_2 emissions (Tables A2, A3). In line with this, the amount of BD showed a significant effect during the regression selection on N_2O, but not on N_2 fluxes (Table 5). A coherent reasoning for the rather smaller emissions of highly amended HIBD treatments might lie in an inhibitory effect of NH_3 on nitrification. Accordingly, Kim et al. (2006) found a selective inhibition of NO_2^- oxidation in the presence of 14 to 17 mg NH_3-N L^{-1}. Our calculated application rates in the treatments with HIBD amounted to at least 253.4 mg NH_4^+-N (kg soil)$^{-1}$ for the clayey silt (Fig. 3), which corresponds to 13.0 mg NH_3-N (kg soil)$^{-1}$ at 15 °C when applying the pH of the BD and assuming all extractable NH_4^+-N to be in solution (Emerson et al., 1975). Hence, we consider this inhibitory effect as the reason for the missing increase in N_2O and N_2. Nevertheless, because we mixed the BD with the soil, one would expect a lower in situ NH_3 fixation by clay minerals in tubular injection slits (Kissel et al., 2008), resulting in probably lower N_2O and N_2 fluxes from clayey soils due to a more marked inhibitory effect.

High NH_4^+ loads in conjunction with an increased pH favour NO_2^- accumulation because NO_2^--oxidizing bacteria are less resilient against high concentrations of NH_3 than NH_3-oxidizing bacteria (Anthonisen et al., 1976). This NO_2^- should have protonated then partly to toxic and unstable HNO_2, which drives biological and chemical production of

NO and N_2O for detoxification (Venterea et al., 2015). Although we did not determine NO_2^-, we suggest a dominant role of nitrifier denitrification, i.e. NO_2^- reduction, in the generation of N_2O during our experiment, especially during the anaerobic headspace conditions at the end of the incubation, resulting in the relatively small NO_3^- recovery in both soils. Accordingly, coupled nitrification–denitrification and bacterial denitrification have been found to dominate the production of N_2O directly after application of BD (Köster et al., 2011; Senbayram et al., 2009). However, N_2O-N losses were clearly larger than N_2 losses under aerobic headspace in the clayey silt. This indicates that much of the N gas loss was driven by processes other than canonical denitrification. Under the above-mentioned conditions, NO-N losses may exceed N_2O losses (Venterea et al., 2015), making it important to take NO measurements into account in future studies.

Supposing that 15 % of NH_4^+-N is volatilized as NH_3 within the first 10 h after surface application of BD (Quakernack et al., 2012), the losses from the NH_4^+ amounts we applied would average to 80 mg NH_3-N m^{-2} h^{-1} (LOBD) and 160 mg H_3-N m^{-2} h^{-1} (HIBD). The actual losses of up to 11.7 mg N_2O-N m^{-2} h^{-1} at 30 % WFPS in the clayey soil (Table A2) or of up to 35.1 mg N_2 m^{-2} h^{-1} at 55 % WFPS in the sandy loam (Table A3) from our HIBD treatments add up to 117 mg N_2O-N and 351 mg N_2, respectively, for the same period. Hence, increased N_2O and N_2 emissions following injection of BD might effectively cause higher N losses compared to a surface application and deserve closer attention in future.

4.2 Different effects of soil diffusivity on N_2O and N_2 fluxes

Apparently, the tested factors affected the N_2O and N_2 fluxes from both soils in a different way. A specific soil characteristic that exhibits such a fundamental control on biogeochemical processes such as denitrification is the diffusivity for O_2 (Ball, 2013; Letey et al., 1980; Parkin and Tiedje, 1984), which is a main soil characteristic responsible for the appearance of anaerobic microsites. In general, diffusivity integrates the soil porosity, i.e. pore continuity and size as well as WFPS, which control both soil N_2O and N_2 emissions (Balaine et al., 2016; Ball, 2013; Letey et al., 1980). Soils with a coarser texture like the loamy sand have a higher proportion of macropores and thus a higher gas diffusion compared with fine-textured soils like the clayey silt we used (Groffman and Tiedje, 1991). This lets us expect conditions that are more favourable for N_2O and N_2 generation in the latter due to relatively poor diffusion characteristics and, thus, a smaller O_2 supply. Actually, although we incubated the soils at comparable levels of WFPS and BD amendments, the apparent lower diffusivity led to larger N_2O and N_2 production in the treatments with the clayey silt in relation to the loamy sand.

The role of the distinct diffusivities of both soils is corroborated by our observations of the gas fluxes in anaerobic headspace. With switching the He–O_2 atmosphere in the headspace to pure He, the denitrification potential can be tested because anaerobicity eliminates respiration processes that use O_2 as an electron acceptor (Parkin and Tiedje, 1984). We acknowledge DNRA and anammox, for example, as possible additional sources of N_2O and N_2 under such conditions but we were not able to quantify their contribution. The anaerobic headspace induced a considerable increase in N_2O fluxes in the loamy sand, but not in the clayey silt. Concurrently, the N_2 fluxes increased in both soils, but pronouncedly, i.e. more than 60-fold, in the sandy loam. These observed changes resulting from oxygen deprivation imply that, during the previous aerobic conditions, the diffusivity of the sandy loam was too high to allow for a sufficient establishment of anaerobic microsites, while the clayey silt ensured a moderate diffusional constraint to maintain suboxic conditions. In general, only N_2O fluxes from treatments with negligible fluxes during the previous aerobic period increased under anaerobic conditions, including all treatments with loamy sand (Fig. 3, Table A2). At the same time, there was a reduction of N_2O fluxes in most clayey silt treatments. However, a closer look reveals that virtually all of the latter treatments showed increased N_2 flux rates. Hence, there was an enhanced reduction of N_2O to N_2, which is reflected in the increased N_2 / (N_2 + N_2O) product ratio (Fig. 5) and points to intensified reduction of N_2O due to the lack of oxygen (Parkin and Tiedje, 1984). The much larger N_2 fluxes from the loamy sand compared to the clayey silt might have been caused as well by poor NO_3^- availability (Fig. 2) and a high availability of C (Table 4), which promoted the reduction of N_2O to N_2 (Senbayram et al., 2012). Further, we found no evidence for any shortage of substrate in the clayey silt during the subsequent anaerobic headspace conditions. However, the cumulated fluxes of both N_2 and N_2O amounted to a maximum absolute loss of 9.4 ($1\sigma = 0.3$) mg N per kg soil in the clayey silt with LOBD and 55 % WFPS, which was 7.4 %

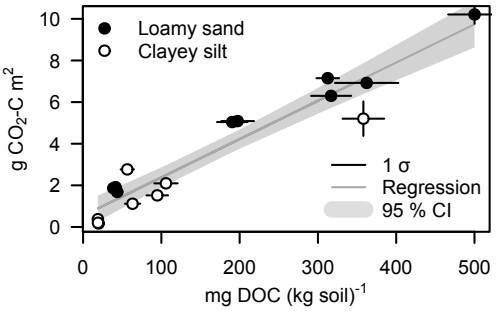

Figure 6. Regression between DOC (mg per 100 g soil) measured after the incubation and the respective cumulated CO_2 emissions (g C m^{-2}) during the period of aerobic headspace with their standard deviations and confidence interval (95 %). If error bars are not visible, they are smaller than the symbols of the means. Both soils showed increasing emissions with increasing soil DOC contents as well as a good regression fit ($R^2 = 0.91$, $p < 0.001$).

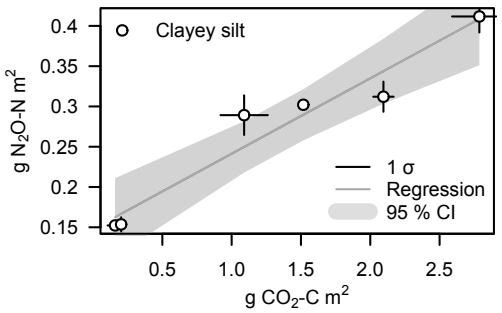

Figure 7. Regression between cumulated CO_2 emissions ($g\,C\,m^{-2}$) and the respective cumulated $N_2O + N_2$ emissions ($g\,N\,m^{-2}$) from the clayey silt with WFPS > 35 % during the period of aerobic headspace with their standard deviations and confidence interval (95 %). If error bars are not visible, they are smaller than the symbols of the means. The proportional increase in CO_2 and the N gas species shows a good regression fit of $R^2 = 0.93$ ($p = 0.001$).

of the calculated NH_4^+-N applied with BD (Fig. 2). Conversely, the N_2 / (N_2+N_2O) product ratios increased only slightly (Fig. 5) and, in contrast to the loamy sand, there were still significant N_2O fluxes in the clayey silt (Fig. 3). This points to still-sufficient stocks of NO_3^- in the latter (Senbayram et al., 2012). In fact, the NO_3^- stock was greater in the clayey silt than in loamy sand after incubation (Fig. 2). Thus, we suggest that the gas fluxes were unaffected by the change to anaerobic headspace in the clayey silt due to already low O_2 concentrations as a result of poor diffusivity. In conclusion, distinct gas diffusivities of both soils can be proposed as the main reason for the differing N_2O and N_2 fluxes.

In interaction with soil diffusivity, respiration affects the aerobicity of a soil matrix by concurrent consumption and formation of O_2 and CO_2 as well. Depending on microbial C availability, respiration could be indicated by DOC, though not all DOC may be readily degradable (Cook and Allan, 1992). Generally, DOC content after our incubation increased with application rate of BD (Table 4), but DOC content was always smaller in clayey silt. This might reflect a stronger sorption of C and thus a lower availability for respiration in the clayey silt compared to loamy sand (Kaiser and Guggenberger, 2000). If we compare DOC concentrations with cumulated flux rates of CO_2 over the period of aerobic headspace, we find a good regression fit ($R^2 = 0.91$, $p < 0.001$) for both soils (Fig. 6), indicating a sufficient availability of C from BD for respiration and, thus, implicitly also for denitrification (Reddy et al., 1982). Moreover, as increased DOC enhanced respiration (Table A1), it consequently affected O_2 consumption and, thus, also the emergence of anaerobic microsites (Azam et al., 2002). Accordingly, there is also a good correlation between cumulated CO_2 and N_2O + N_2 fluxes for the same period from the clayey silt ($R^2 = 0.93$, $p = 0.001$), when the treatments with 35 % WFPS (which showed virtually no N emissions) are omitted (Fig. 7). However, there was no such a correlation for the loamy sand. This confirms the interactive effect of diffusivity (induced by both the soils and WFPS) and C availability on the emissions of N_2O and N_2, which, nevertheless, interacted with the inhibitory effect of high NH_4^+ loads on nitrification (see Sect. 4.1).

5 Relevance and implications

Our aim was to estimate the effect of differing soil environmental conditions on gaseous N losses – and not to draw conclusions about the long-term dynamics of N_2 and N_2O emissions after BD application in concentrations similar to injection. In another laboratory study at a WFPS of 65 %, Senbayram et al. (2009) measured only one peak within 2 days without a repeated increase later, regardless of the amount of applied BD. Thus, we assume that a single peak shortly after application holds also true for our incubation. We also assume the measurements after only 24 h of anaerobicity in the headspace to be representative for the emission potential since in similar studies Wang et al. (2011, 2013) showed that the emission of N_2 and N_2O peaked within less than 24 h after switching their headspace from aerobic to anaerobic conditions.

In summary, as hypothesized, N_2O and N_2 emissions as well as the N_2 / (N_2O+N_2) product ratio increased with WFPS, most probably due to restricted supply of O_2. Contrary to our second hypothesis, the gaseous losses of N_2O and N_2 did not increase with the application rate of BD. This indicates an inhibitory effect of high NH_3 and NH_4^+ concentrations on nitrification, which are typically found in BD. At the same time, the N_2 / (N_2O+N_2) product ratio tended to decrease with application rate as supposed, probably due to a copious supply with NO_2^- and NO_3^- from oxidized BD-NH_4^+. Confirming our third hypothesis, the fine textured clayey silt induced larger gaseous N losses and a higher N_2 / (N_2O+N_2) ratio than the coarse loamy sand by the apparent distinct diffusivities of both soils. Overall, there was a larger potential for formation of N_2O in the fine-textured clayey silt compared to the coarse loamy sand after the application of high concentrations of BD as they may appear after injection. However, the loamy sand showed a large potential for N_2 formation under anaerobic headspace conditions.

Since coupled nitrification–denitrification N losses from injected BD seem to be massive in this study, the short-term emissions of N_2O and N_2 after injection appear to offset the reduced NH_3-N losses that would have hypothetically arisen from surface application. Further investigations are needed regarding the dynamics and the duration of the observed effects and their reliability for field conditions.

Data availability. The data underlying the figures are accessible publicly via the Supplement.

Appendix A

Table A1. Mean CO_2-C fluxes with standard deviations in mg m^{-2} h^{-1} from the loamy sand and the clayey silt, treated with different water-filled pore spaces (WFPSs, %), amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD) and different temperature regimes (°C) under aerobic (He–O_2) and anaerobic (He) atmospheres. The column "Day" denotes the consecutive measuring days of the respective incubation cycle. Different letters after fluxes indicate significant differences (Tukey's HSD, $p < 0.05$) within each soil and measuring day. Zeros as last digits were omitted.

Day	Atmosphere	Temperature (°C)	WFPS (%)		mg CO_2-C m^{-2} h^{-1}	
					Loamy sand	Clayey silt
1	He–O_2	2	35	No BD	6.8 ± 2.4 cd	0 ± 0 c
1	He–O_2	2	35	Low BD	22 ± 3.5 bcd	NA
1	He–O_2	2	35	High BD	23.3 ± 9.3 bc	22.8 ± 2.8 ab
1	He–O_2	2	55	No BD	6 ± 0.7 d	4.6 ± 7.9 bc
1	He–O_2	2	55	Low BD	34.4 ± 3.1 b	34.5 ± 11.6 a
1	He–O_2	2	55	High BD	28 ± 3.2 b	15.9 ± 3.4 abc
1	He–O_2	2	75	No BD	9.4 ± 1.4 cd	0 ± 0 c
1	He–O_2	2	75	Low BD	37.5 ± 6 b	15.5 ± 12.1 abc
1	He–O_2	2	75	High BD	68.3 ± 12.1 a	24.5 ± 2.7 a
2	He–O_2	2	35	No BD	9.8 ± 3.5 c	1.3 ± 1.4 b
2	He–O_2	2	35	Low BD	23 ± 3.9 bc	NA
2	He–O_2	2	35	High BD	30.9 ± 2.2 b	22.2 ± 2.4 a
2	He–O_2	2	55	No BD	8.7 ± 1.5 c	0.6 ± 1 b
2	He–O_2	2	55	Low BD	33.4 ± 0.9 b	27.6 ± 12.3 a
2	He–O_2	2	55	High BD	35.9 ± 2.7 b	14.4 ± 1.9 ab
2	He–O_2	2	75	No BD	8.3 ± 1.5 c	0 ± 0 b
2	He–O_2	2	75	Low BD	31.9 ± 3 b	13 ± 9.3 ab
2	He–O_2	2	75	High BD	57.6 ± 14.8 a	18.3 ± 4 a
3	He–O_2	15	35	No BD	42.5 ± 4.5 c	6.7 ± 0.7 b
3	He–O_2	15	35	Low BD	114.3 ± 12.2 b	NA
3	He–O_2	15	35	High BD	149.5 ± 9.4 b	130.9 ± 105 a
3	He–O_2	15	55	No BD	41.3 ± 3.5 c	3.2 ± 0.4 b
3	He–O_2	15	55	Low BD	108.7 ± 10.1 b	57.8 ± 12.2 bc
3	He–O_2	15	55	High BD	162.1 ± 9.6 b	26.8 ± 0.7 bc
3	He–O_2	15	75	No BD	44.1 ± 9.8 c	3.2 ± 0.7 b
3	He–O_2	15	75	Low BD	150.4 ± 19 b	26.4 ± 11.8 bc
3	He–O_2	15	75	High BD	249.7 ± 53.5 a	35.3 ± 6 bc
4	He–O_2	15	35	0	48.7 ± 6 c	15.1 ± 4.9 cd
4	He–O_2	15	35	Low BD	114.3 ± 6.4 b	NA
4	He–O_2	15	35	High BD	156.9 ± 15.4 a	65.7 ± 2.2 a
4	He–O_2	15	55	No BD	48 ± 3.4 c	4.2 ± 0.2 d
4	He–O_2	15	55	Low BD	109 ± 14.4 b	51.2 ± 15.1 ab
4	He–O_2	15	55	High BD	177.7 ± 7.5 a	26.6 ± 2.3 cd
4	He–O_2	15	75	No BD	34 ± 7.8 c	6.7 ± 4 d
4	He–O_2	15	75	Low BD	168.7 ± 0.4 a	22.1 ± 14.8 cd
4	He–O_2	15	75	High BD	166.3 ± 23.1 a	34.1 ± 5.7 bc
5	He	15	35	No BD	11.2 ± 0.6 d	NA
5	He	15	35	Low BD	54.8 ± 9.3 c	NA
5	He	15	35	High BD	149.3 ± 3.9 a	45.8 ± 2.1 a
5	He	15	55	No BD	13.6 ± 1.9 d	3.4 ± 0.6 c
5	He	15	55	Low BD	55.2 ± 4.4 bc	32 ± 11.4 ab
5	He	15	55	High BD	164.5 ± 3.5 a	15.2 ± 10.7 bc
5	He	15	75	No BD	20.9 ± 2.3 d	3.6 ± 0.1 c
5	He	15	75	Low BD	75 ± 7.3 b	20.6 ± 8.5 bc
5	He	15	75	High BD	NA	26.1 ± 2.6 ab

Table A2. Mean N_2O-N fluxes with standard deviations in mg m^{-2} h^{-1} from the loamy sand and the clayey silt, treated with different water-filled pore spaces (WFPSs, %), amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD) and different temperature regimes (°C) under aerobic (He–O_2) and anaerobic (He) atmospheres. The column "Day" denotes the consecutive measuring days of the respective incubation cycle. Different letters after fluxes indicate significant differences (Tukey's HSD, $p < 0.05$) within each soil and measuring day. Zeros as last digits were omitted.

Day	Atmosphere	Temperature (°C)	WFPS (%)		mg N_2O-N m^{-2} h^{-1}	
					Loamy sand	Clayey silt
1	He–O_2	2	35	No BD	0 ± 0	0 ± 0 c
1	He–O_2	2	35	Low BD	0 ± 0	NA
1	He–O_2	2	35	High BD	0 ± 0	0 ± 0 c
1	He–O_2	2	55	No BD	0 ± 0	0.3 ± 0.1 c
1	He–O_2	2	55	Low BD	0 ± 0	1.7 ± 0.4 a
1	He–O_2	2	55	High BD	0 ± 0	1.1 ± 0.1 b
1	He–O_2	2	75	No BD	0 ± 0	0.4 ± 0.1 c
1	He–O_2	2	75	Low BD	0 ± 0	1 ± 0.1 b
1	He–O_2	2	75	High BD	0 ± 0	1 ± 0.2 b
2	He–O_2	2	35	No BD	0 ± 0	0 ± 0 d
2	He–O_2	2	35	Low BD	0 ± 0	NA
2	He–O_2	2	35	High BD	0 ± 0	0 ± 0 cd
2	He–O_2	2	55	No BD	0 ± 0	0.3 ± 0.1 bc
2	He–O_2	2	55	Low BD	0 ± 0	1.5 ± 0.6 a
2	He–O_2	2	55	High BD	0 ± 0	1.2 ± 0.2 a
2	He–O_2	2	75	No BD	0 ± 0	0.4 ± 0.1 bc
2	He–O_2	2	75	Low BD	0 ± 0	1 ± 0.1 ab
2	He–O_2	2	75	High BD	0 ± 0	1.1 ± 0.2 a
3	He–O_2	15	35	No BD	0 ± 0 cd	0 ± 0 c
3	He–O_2	15	35	Low BD	0 ± 0 abc	NA
3	He–O_2	15	35	High BD	0 ± 0 ab	0 ± 0 c
3	He–O_2	15	55	No BD	0 ± 0 bcd	0.8 ± 0.2 c
3	He–O_2	15	55	Low BD	0 ± 0 bcd	7.1 ± 0.9 a
3	He–O_2	15	55	High BD	0 ± 0 a	3.5 ± 0.7 b
3	He–O_2	15	75	No BD	0 ± 0 ab	0.8 ± 0.2 c
3	He–O_2	15	75	Low BD	0 ± 0 d	3.2 ± 0.7 b
3	He–O_2	15	75	High BD	0 ± 0 cd	3 ± 0.9 b
4	He–O_2	15	35	No BD	0 ± 0 b	0 ± 0 c
4	He–O_2	15	35	Low BD	0 ± 0 ab	NA
4	He–O_2	15	35	High BD	0 ± 0 ab	0.1 ± 0.1 c
4	He–O_2	15	55	No BD	0 ± 0 b	1 ± 0.2 bc
4	He–O_2	15	55	Low BD	0.1 ± 0.1 a	6.2 ± 1.1 a
4	He–O_2	15	55	High BD	0 ± 0 ab	3 ± 0.8 b
4	He–O_2	15	75	No BD	0 ± 0 ab	1.1 ± 0.3 bc
4	He–O_2	15	75	Low BD	0 ± 0 b	2.6 ± 1 b
4	He–O_2	15	75	High BD	0 ± 0 b	2.2 ± 0.9 b
5	He	15	35	No BD	0.1 ± 0	NA
5	He	15	35	Low BD	NA	NA
5	He	15	35	High BD	0.9 ± 0.1	11.7 ± 2 a
5	He	15	55	No BD	0.1 ± 0	0.1 ± 0 c
5	He	15	55	Low BD	NA	5 ± 1 b
5	He	15	55	High BD	1.2 ± 0.7	1.4 ± 0.8 c
5	He	15	75	No BD	1.3 ± 1.6	0.1 ± 0 c
5	He	15	75	Low BD	NA	1.7 ± 0.3 c
5	He	15	75	High BD	NA	1 ± 0.3 c

Table A3. Mean N_2 fluxes with standard deviations in mg m^{-2} h^{-1} from the loamy sand and the clayey silt, treated with different water-filled pore spaces (WFPSs, %), amounts of digestate (0 mL per sample ring: no BD, 17.6 mL: low BD and 35.2 mL: high BD) and different temperature regimes (°C) under aerobic (He–O_2) and anaerobic (He) atmospheres. The column "Day" denotes the consecutive measuring days of the respective incubation cycle. Different letters after fluxes indicate significant differences (Tukey's HSD, $p < 0.05$) within each soil and measuring day. Zeros as last digits were omitted.

	Atmosphere	Temperature (°C)	WFPS (%)		mg N_2 m^{-2},h^{-1}	
					Loamy sand	Clayey silt
1	He–O_2	2	35	No BD	0 ± 0	0 ± 0 bc
1	He–O_2	2	35	Low BD	0 ± 0	NA
1	He–O_2	2	35	High BD	0 ± 0	0.1 ± 0.1 bc
1	He–O_2	2	55	No BD	0 ± 0	1.5 ± 0.3 a
1	He–O_2	2	55	Low BD	0 ± 0	1.5 ± 0.3 a
1	He–O_2	2	55	High BD	0 ± 0	1.5 ± 0 a
1	He–O_2	2	75	No BD	0 ± 0	1.2 ± 1.1 a
1	He–O_2	2	75	Low BD	0 ± 0	1.5 ± 0.2 a
1	He–O_2	2	75	High BD	0 ± 0	1.7 ± 0.4 a
2	He–O_2	2	35	No BD	0 ± 0	0 ± 0 c
2	He–O_2	2	35	Low BD	0 ± 0	NA
2	He–O_2	2	35	High BD	0 ± 0	0 ± 0 c
2	He–O_2	2	55	No BD	0 ± 0	1.3 ± 0.1 a
2	He–O_2	2	55	Low BD	0 ± 0	0.8 ± 0.5 b
2	He–O_2	2	55	High BD	0 ± 0	1.3 ± 0.3 a
2	He–O_2	2	75	No BD	0 ± 0	1.4 ± 0.4 a
2	He–O_2	2	75	Low BD	0 ± 0	1.4 ± 0.5 a
2	He–O_2	2	75	High BD	0 ± 0	1.4 ± 0.1 a
3	He–O_2	15	35	No BD	0 ± 0 b	0 ± 0 e
3	He–O_2	15	35	Low BD	0 ± 0 b	NA
3	He–O_2	15	35	High BD	0.1 ± 0.1 ab	0 ± 0 e
3	He–O_2	15	55	No BD	0 ± 0 b	1.8 ± 0.3 cd
3	He–O_2	15	55	Low BD	0 ± 0 b	2.3 ± 0.4 bc
3	He–O_2	15	55	High BD	0 ± 0 b	2.5 ± 0.2 ab
3	He–O_2	15	75	No BD	0.2 ± 0.3 a	1.5 ± 0.2 d
3	He–O_2	15	75	Low BD	0 ± 0 b	3 ± 0.9 a
3	He–O_2	15	75	High BD	0 ± 0 b	2.6 ± 0.8 ab
4	He–O_2	15	35	No BD	0 ± 0 b	0 ± 0 c
4	He–O_2	15	35	Low BD	0.1 ± 0.2 b	NA
4	He–O_2	15	35	High BD	0 ± 0 b	0 ± 0 c
4	He–O_2	15	55	No BD	0.1 ± 0.1 b	1.5 ± 0.2 b
4	He–O_2	15	55	Low BD	0 ± 0 b	2.9 ± 0.6 a
4	He–O_2	15	55	High BD	0.5 ± 0.4 a	2.8 ± 0.7 a
4	He–O_2	15	75	No BD	0 ± 0 b	1.3 ± 0.2 bc
4	He–O_2	15	75	Low BD	0 ± 0 b	3.8 ± 1.6 a
4	He–O_2	15	75	High BD	0 ± 0 b	3.3 ± 0.8 a
5	He	15	35	No BD	3.3 ± 0.4 d	0 ± 0 c
5	He	15	35	Low BD	NA	NA
5	He	15	35	High BD	22.9 ± 5.7 b	1.8 ± 0.1 c
5	He	15	55	No BD	6 ± 2.2 cd	1.8 ± 0.2
5	He	15	55	Low BD	NA	9.5 ± 2.7 a
5	He	15	55	High BD	35.1 ± 8.6 a	5.1 ± 1.8 bc
5	He	15	75	No BD	9.2 ± 0.4 c	1.9 ± 0.1 c
5	He	15	75	Low BD	NA	4.8 ± 1.6 bc
5	He	15	75	High BD	26.8 ± 1.1 b	6.7 ± 0.8 b

The Supplement related to this article is available online at https://doi.org/10.5194/soil-3-161-2017-supplement.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. We thank the editor Karsten Kalbitz and the three anonymous referees for their careful reading, critical comments and valuable suggestions. We are very grateful to Heinrich Graf von Bassewitz and Matthias Haß from Gut Dalwitz for their straightforward support with substrate from their anaerobic digester. We thank Madlen Pohl from the ZALF, Institute for Landscape Biogeochemistry, Müncheberg, Germany, most sincerely for managing the laboratory analyses of the soil samples. The joint research project underlying this report was funded by the German Federal Ministry of Food and Agriculture under the funding identifier 22007910.

Edited by: Karsten Kalbitz
Reviewed by: three anonymous referees

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5 General discussion

5.1 Short-term impact of tillage

5.1.1 Lower microbial availability of C from BD amendment affects soil respiration

The application of BD as a soil amendment has become a common practice due to the promotion of biogas plants during the last two decades, especially in Germany. Although it is an organic fertiliser, BD contains less organic C and an increased proportion of NH_4^+ and has a higher pH than the undigested precursors (Möller and Müller, 2012; Möller, 2015). Regarding long-term applicability, this altered composition has been questioned to be coherent with a sustainable and environmentally sound agriculture in terms of soil fertility or the volatilisation of NH_3 and the GHG N_2O (Möller, 2015).
Principally, the organic fraction of BD is smaller and more recalcitrant than the input feedstock due to the mineralisation processes during AD. In fact, the organic matter, which is relocated to the soil by BD amendment, has a reduced content of easily degradable C (Askri et al., 2015; Alburquerque et al., 2012a; Coban et al., 2015) but simultaneously an increased share of potential humus precursors like complex lipids, lignin-like material and steroids (Lorenz et al., 2007). It is suggested, that slower growing microorganisms (*K*-strategists) with a reduced growth rate and activity will establish consequently in the soil (Chen et al., 2012), which result in a slower SOM turnover (Bachmann et al., 2014; Eichler-Löbermann et al., 2014; Johansen et al., 2013). Hence, a similar SOM reproduction from the stable organic fractions of digested and undigested amendments is anticipated (Figure 5-1). In other words, the microbial available C fractions, which are converted into biogas during AD, are principally correlated to the fractions of undigested organic matter, which are preferably decomposed by microorganisms in the soil (Möller, 2015; Insam et al., 2015; Thomsen et al., 2013). Overall, recent studies assume no compromising effect on soil fertility after a regular application of BD.

5.1.2 Lower immediate CO_2 efflux after tillage possibly due to higher diffusivity of BD amended soils

The immediate efflux of CO_2 from broken up soil pores showed different magnitudes directly after tillage (Fiedler et al., 2015). One could hypothesise that the supposed general lower levels of respiration in the BD treatments caused a lower input of CO_2 for storage in soil pores. However, the pore size distribution and the pore connectivity may exhibit a much higher influence on the storage capacity. Evidently, organic soil amendments improve the amount of macropores and the pore-network connectivity (Schlüter et al., 2011). This holds probably also true for the use of BD since the pure BD treatments of the present long-term study site showed in some phases of the crop rotation (e.g. during the stand of triticale 2011−2012) a significantly lower mean bulk density of 1.44 g cm^{-3} than the corresponding treatment with mineral fertiliser (1.60 g cm^{-3}). In accordance, Garg et al. (2005) observed a similar reduction of bulk density and, simultaneously, an increase of the water retention capacity of a soil amended with BD. However, at the first sight, an increased amount of macropores may enhance the gas storage capacity. But in association with an implicitly higher degree of pore connectivity, the hereby improved diffusivity facilitates also gas exchange with the atmosphere along a concentration and pressure gradient by diffusion and advection, respectively (Ball, 2013; Moldrup et al., 2001; van der Weerden, Tony J. et al., 2012). Thus, a lower storage capacity for CO_2 is anticipated here as a reason for the lower magnitudes of the initial CO_2 efflux from the BD treatments after tillage. Further studies should focus on the impact of BD amendment on gas specific physical soil properties to clarify this issue.

5.1.3 Lower increase of soil respiration after tillage due to lower C availability and antimicrobial compounds from BD – but conversely, significant turnover of lignin derived compounds

The assumption of a limited microbial activity due to a lower amount of readily available C in soils amended with BD is certainly also the reason for the observed effects on SR after tillage in the present studies. Tillage disrupts soil macroaggregates and exposes previously protected SOM to microbial decomposition (Six et al., 2000; Rochette and Angers, 1999). The increased proportion of lipids at the expense of carbohydrates and peptides found by Fiedler et al. (2016) in the mineral fertilised treatment confirms the concomitant promotion of microbial activity after tillage. If, however, this *de novo* ex-

posed SOM is composed of less readily decomposable C, then the immediate mineralisation rates of this SOM should be lower than from entities with a relatively high share readily decomposable C. Indeed, the present comparison of tillage effects approved this hypothesis. In the treatments with BD, the increase of respiration was much lower after tillage compared to the other treatments, either with mineral or without fertiliser, which point to the role of C availability as a substrate for microbial respiration (Fiedler et al., 2015; Fiedler et al., 2016). Accordingly, the observed short-term decomposition of lignin and the simultaneous formation of microbial carbohydrates and peptides point to the utilisation of lignin-derived moieties and lipids by microorganisms at low initial availability of C as suggested by Kalbitz et al. (2003).

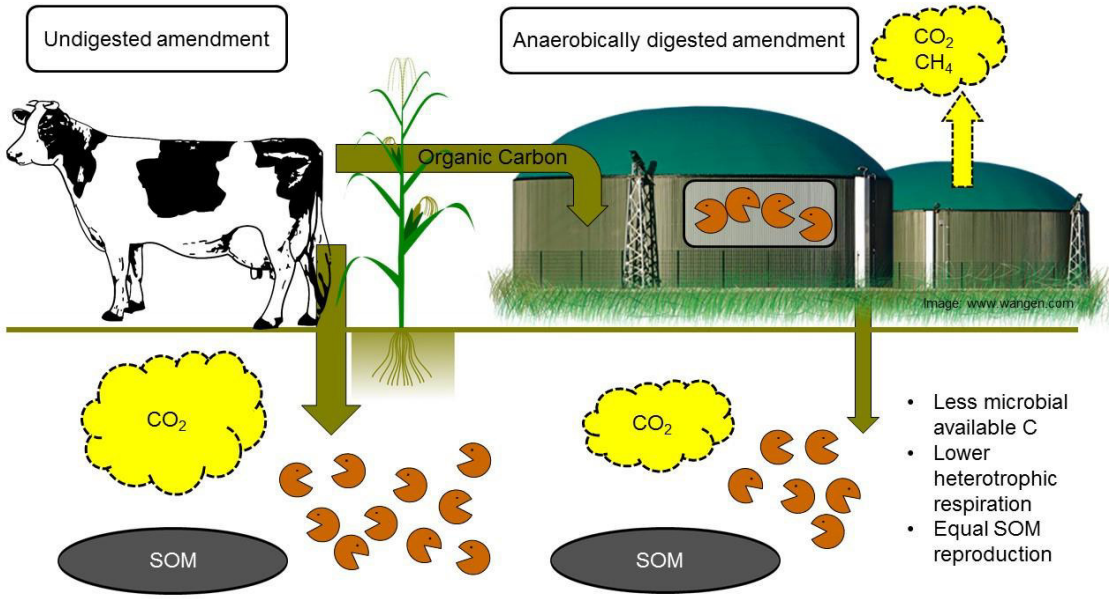

Figure 5-1. Effect of anaerobically digested amendment on microbial C availability, heterotrophic respiration and SOM reproduction. Own presentation.

Nevertheless, other causes might have contributed to the less pronounced increase of respiration as well. According to our results, also a hampering effect of specific BD compounds (e.g. sterols) on microbial activity could be assumed, especially of *β*-sitosterol on microbes related to N mineralisation (Heumann et al., 2013; Heumann et al., 2011). There might be further enrichments of specific antimicrobial compounds, which have to be studied more extensively. For example, specific phenols from AD of pig manure were identified to hamper NH_3 oxidisers (Levén et al., 2006; Nyberg et al., 2004), though, they are not accountable directly for CO_2 production. However, autotrophic nitrifiers are consuming CO_2 (Wrage et al., 2001), hence their hampering may even increase the partial pressure of CO_2 in the soil.

5.1.4 Shorter and less pronounced disturbance of model calibration after tillage in BD treatments

Regarding the calibration with of Arrhenius type model (Eq. 1-1) by Lloyd and Taylor (1994) for SR, tillage generally disturbed explanatory power of temperature abruptly (Fiedler et al., 2015). Additionally, the influence of other factors – which are not considered in Eq. (1-1) – may increase. Presumably, the effect of soil moisture (i.e. drought) was strengthened after tillage since tilled soils generally have a lower water holding capacity than non-tilled soils (Nyborg and Malhi, 1989; Zhang et al., 2011). Directly after tillage, the soil aeration increases and water, which was stored in pores without connection to the atmosphere, is exposed to evaporation due to soil comminution. Simultaneously, the recently tilled soil has a reduced water holding capacity (WHC) and is prone to desiccation in the upper soil layer for the same reason. Thus, water may have become the overriding and most limiting factor for SR. In general, soil moisture extremes can prevent the temperature response of microbial activity (Davidson et al., 2012). Further, the disturbance of temperature driven SR model lasted shorter in the BD treatment. This behaviour could be ascribed probably also to a less vulnerable WHC and to the lower increase of microbial available C after tillage. Generally, the application of BD can improve the water retention capacity of soils (Garg et al., 2005), which could be attributed to a greater amount of SOC (Rawls et al., 2003) and SOM (Hudson, 1994), respectively, and a generally better aggregation (Grandy et al., 2002) of organically amended soils. The latter assumptions seem to hold true also for BD since a similar SOM reproduction is assumed in comparison to undigested organic amendments (see section 5.1.1). Accordingly, the constantly lower values for E_0 of the SR model for the BD treatment and their high variance in the mineral treatments (Fiedler et al., 2015) support the assumption that the temperature-dependence was disturbed by water limitation (Davidson and Janssens, 2006).

5.2 Short-term impact of high concentrated biogas digestate

5.2.1 Do N_2O and N_2 emissions after injection level off NH_3-N losses after surface application of BD?

Beside N_2O production by spring thaw and after tillage, the first week after the application of fertiliser is associated with the highest flux rates from arable soils (Drewer et al.,

2017; Kaiser and Heinemeyer, 1996; Dobbie et al., 1999). Although N_2O emissions seem to be lower rather than higher due to AD, the increased pH and NH_4^+ content of BD, however, evidently promote NH_3 emissions after BD application, at least during the first hours after surface application (Möller and Stinner, 2009; Quakernack et al., 2012). To reduce NH_3 losses, the application of BD by injection is recommended, but this measure can simultaneously promote conditions favourable for denitrification, i.e. anaerobic microsites (Wulf et al., 2002; Webb et al., 2010). Nitrification of the high NH_4^+ loads from freshly incorporated BD is accompanied by significant consumption of O_2 (Christensen and Rowe, 1984), which is further depleted by microbial respiration of the co-occurring C supply (Dell et al., 2011). The hence fostered anaerobic conditions in the injection band – together with the diffusion path to the soil surface – might result in significant emissions of N_2O and N_2 (Köster et al., 2015; Webb et al., 2010). However, it was unclear how denitrification will respond to such high concentrations of BD as they might appear in injection bands.

The results by Fiedler et al. (2017) indicate that high nutrient concentrations could indeed fuel coupled nitrification–denitrification and nitrifier denitrification with N_2O and N_2 losses exceeding the assumed reductions of NH_3 emissions from injection. By reproducing a BD concentration occurring hypothetically in injection slits with a spacing of 30 cm after an application rate of 160 kg N ha^{-1}, Fiedler et al. (2017) calculated losses of up to 117 mg N_2O-N m^{-2} from a clayey silt or of up to 351 mg N_2 m^{-2} from a sandy loam for a period of ten hours. According to Quakernack et al. (2012), 15 % of NH_4^+-N could be volatilised as NH_3 within the first ten hours after surface application, resulting in a hypothetical reduction of 160 mg NH_3-N in the present study. If one considers also the probably high NO emissions, the gaseous N losses from highly concentrated BD exceed very likely the assumed reduction of NH_3-N losses. Moreover, since increased N_2O and N_2 emissions after injection are supposed to last for at least for a few days (Askri et al., 2015; Köster et al., 2015), even the cumulated N_2O loss after injection by itself may be higher than the impeded NH_3 emissions. However, an incubation experiment is artificial per se and the present one did not study either the emission dynamics or the NH_3 losses. Nevertheless, the here presented results indicate reasonable doubts about the environmental benefit of BD injection and warrant further studies on the emission dynamics, including a direct comparison of impeded NH_3–N losses against those of N_2O, NO and N_2.

5.2.2 High NH_4^+ concentrations probably hamper N_2O and N_2 emissions after the application of increasing amounts of BD and may promote NO-N losses

The results of Fiedler et al. (2017) also showed that high NH_4^+ concentrations (as suggested to appear in injection bands of BD) may result in a restricted nitrification, because no differences in the emissions of N_2O and N_2 were observed after a doubling of the BD concentration from 17.6 to 35.2 mL per soil core (250 cm³). These amounts correspond to 14 (sandy loam) and 13 (clayey silt) mg NH_3-N (kg soil)$^{-1}$ at 15 °C when applying the pH of the BD and assuming all extractable NH_4^+-N to be in solution (Emerson et al., 1975). Accordingly, Kim et al. (2006) found a selective inhibition of NO_2^- oxidation in the presence of 14 to 17 mg NH_3-N L^{-1}, which strengthens the assumption that NO_2^--oxidising bacteria are less resilient against high concentrations of NH_3- than NH_3-oxidising bacteria (Anthonisen et al., 1976). Moreover, the activity of NO_2^--oxidising bacteria was observed to decrease more than 50 % already at 0.7 mg NH_3-N L^{-1} (Kim et al., 2006). Hence an accumulation of NO_2^- is suggested in the alkaline milieu of BD, which in turn is converted to NO and N_2O for detoxification (Venterea et al., 2015). Under such conditions, NO-N emissions may exceed N_2O-N emissions (Venterea et al., 2015), which demand the additional accounting of NO in future studies to estimate the actual gaseous losses of N after the application of BD. One could even hypothesise that the resulting NO emissions from NO_2^- reduction may counteract the potential NH_3 reductions achieved by injection since NO and its successor from oxidation in the atmosphere (nitrogen dioxide, NO_2) contribute likewise to acidification and acidification of ecosystems (Bouwman et al., 2002).

One should consider that Fiedler et al. (2017) mixed the BD into both soils. This procedure could have increased the surface area for potential adsorption of NH_3 on clay minerals (Kissel et al., 2008), resulting in a maybe lower inhibition of nitrification during the laboratory study compared to the expected situation inside tubular injection bands consisting of pure BD *in situ*. Overall, to classify gaseous N emissions from high concentrations of BD, a direct comparison with the same N equivalents of undigested amendment is required, including emission determination of NH_3, N_2O, NO and N_2.

5.2.3 Fundamentally dependence of N_2O and N_2 emissions on soil diffusivity

The study of Fiedler et al. (2017) revealed also the fundamental role of soil gas diffusivity on both the magnitudes and the proportions of N_2O and N_2 emissions after the appli-

cation of BD. Together with WFPS, porosity determines the actual gas diffusivity through soil air and hence the possibility for the formation of anoxic sites favourable for denitrification (Balaine et al., 2016; Ball, 2013; Letey et al., 1980). The relative soil gas diffusivity can be described with a generalised density-corrected model by Deepagoda et al. (2011) (Eq. 5-1):

$$\frac{D_p}{D_0} = 0.5\varphi(\frac{\varepsilon}{\varphi})^3, \quad (5\text{-}1)$$

where D_P and D_0 are the diffusion coefficients in soil and free air, respectively, and ε is air-filled porosity.

The possibility and the length of a diffusion pathway in the gaseous phase (i.e. soil tortuosity) is particularly controlled by the connectivity of air-filled pores (Rabot et al., 2015). Water creates menisci in pores, which discontinue this diffusion pathways and increase the tortuosity of wet soils, especially at high water contents (Moldrup et al., 2001). Since WFPS does not account for the effect of these parameters on soil gas diffusivity, different soil types do not have necessarily the same aeration status at an identical WFPS, as demonstrated in the present study. However, these parameters are hardly to estimate but depend in uncompacted soils basically on ρ_b, which is a function of soil texture and SOM content (Keller and Håkansson, 2010).

A second restraint for O_2 supply into soil is the much lower solubility of O_2 in water compared to that of N_2O (Heincke and Kaupenjohann, 1999). Nevertheless, diffusion of N_2O in water is reduced by four orders of magnitude compared to its diffusion in air. At the same time, however, the temporal storage of N_2O in soil water increases the time for its potential reduction to N_2 (Clough et al., 2005; Heincke and Kaupenjohann, 1999). Apparently, the clayey silt constrained the diffusional O_2 supply, resulting in enhanced N_2 emissions and a larger N_2 / (N_2O+N_2) product ratio with increasing WFPS. To the contrary, the loamy sand was much more diffusive, which was indicated by mostly negligible N_2O and N_2 emissions during the period of aerobic headspace, independent of WFPS. This differing behaviour can be explained by differences in porosity and pore size distribution of both soils, resulting from their individual texture. Sandy soils with their coarse structure have a much higher proportion of macropores compared to silty soils. The latter are characterised by a predominance of micropores (Groffman and Tiedje, 1991). Along with a larger proportion of macropores, the share of drained and connected pores increases, which enable an easier gas diffusion (van der Weerden, Tony J.

et al., 2012; Moldrup et al., 2001). Hence pore size distribution affects O_2 availability, which WFPS does not account for (Zhu et al., 2013).

Nevertheless, the loamy sand emitted substantially increased amounts of N_2 during the following period of anaerobic headspace. This emission behaviour is reasoned by the fact, on the one hand, that the abundance of denitrifiers is relatively widespread in soils. They account for up to 6 % of the bacterial community – albeit more than a fifth of these denitrifiers do not possess the *nosZ* gene, which encodes the only known enzyme for N_2O reduction to N_2, i.e. N_2O reductase (Henry et al., 2006). The short-term appearance of N_2 emissions, on the other hand, is reasonable since most denitrifiers are facultative anaerobes, which can switch quickly from aerobic respiration of O_2 to anaerobic respiration of nitrogen oxides, when the availability of O_2 as terminal electron acceptor gets insufficient (Tiedje, 1988; Rudaz et al., 1991). Both the abundance and the quick reaction of denitrifiers on anaerobic headspace conditions, verify the assumption of a sufficient diffusivity for O_2 supply in the sandy loam during the previous aerobic headspace. To the contrary, the clayey silt emitted only slightly higher fluxes with a subtle increased N_2 / (N_2O+N_2) product ratio after the headspace has been switched to anaerobic conditions. This behaviour points to an already constrained diffusion of O_2 in the clayey silt before the headspace changed. The present results demonstrate that the effect of soil texture on soil gas diffusivity can override the effect of WFPS and nutrient concentrations on N_2O and N_2 emissions (Klefoth et al., 2014)

5.2.4 Reliability of the helium-oxygen method for quantification of N_2 fluxes

In general, the quantification of N_2 is very difficult due to its high background concentration in the atmosphere (Groffman et al., 2006). Generally, there still exist only two appropriate methods to tackle this challenge: ^{15}N labelling and the use of an N_2 free artificial headspace (Lewicka-Szczebak et al., 2017). Up to now, both methods – used separately or in combination – are only applicable together with laboratory incubations. Recent efforts to determine N_2 fluxes on field sites confirmed the need to continue the development of an *in situ* method, which may allow for an indirect estimation of N_2 emissions by a combination of field $^{15}N_2O$ measurements and incubations for $^{15}N_2$ / ($^{15}N_2O$+$^{15}N_2$) determination (Lewicka-Szczebak et al., 2017). Since BD is a rather heterogeneous substrate (Andruschkewitsch et al., 2013), ^{15}N labelling of NH_4^+ may end up in a relative uneven distribution of the latter. Nevertheless, to establish a device for

the helium–oxygen method is also a protracted issue due to the efforts needed to achieve complete tightness against contamination from air born N_2. However, one should consider that the findings by Fiedler et al. (2017) represent rather a first snap shot of the processes involved directly after the application of high concentrated BD and do not allow for conclusions about the following dynamics due to the methodically conditioned short incubation time of a few days. Despite that, similar studies observed N_2O emission peaks within the first two days (e.g. Senbayram et al., 2009), which give point to the measured emissions by Fiedler et al. (2017). Such a period of few days should be also sufficient to record the concomitant N_2 peaks since microbes associated with the production of N_2O and N_2 in soils are able to react fast to changing environmental conditions by utilising existing enzymes within minutes or by *de novo* synthesis within 4–8 hours (Rudaz et al., 1991). Thus, at least the proportions – i.e. the N_2O / (N_2O+N_2) product ratio obtained by Fiedler et al. (2017) – may be used to extrapolate N_2 losses to field situations.

Summary and Outlook

The present studies revealed that the impact of BD on biogeochemical C and N cycling processes with their links to GHG emissions and gaseous nutrient losses are a reasonable object of investigation. Indeed, BD amended soil treatments revealed a distinct CO_2 emission pattern compared to unamended and mineral fertilised treatments during the days after tillage. Immediately after tillage, the BD treatments did not show a pronounced burst of physically degassed CO_2 as observed in the other treatments, perhaps because of a lower capacity for gas storage in such organically amended soils, resulting from an increased macroporosity, which promotes diffusion pathways. However, this hypothesis has to be examined by further research. Evidently, the increase in SR due to tillage-induced mineralisation by macroaggregate comminution was significantly lower in BD treatments. Most probably, the restricted availability of readily biodegradable C compounds from the amendment with BD hampered the additional respiration activity after tillage. The coincidently reduction of microbial activity by anti-microbial substances like sterols could have been an additional treat to SR.

The distinct behaviour of BD treatments was also reflected in the impact of tillage on the recalibration of temperature-driven models for SR. The decreased validity of temperature dependence was shorter and less strongly pronounced in BD amended soils compared to the other treatments. In the latter, a poor predictive significance and a sug-

gested increase of sensitivity for other variables like soil moisture were observed for at least two weeks after a tillage event in late summer. Presumably, the organic amendment with BD stabilised the soil aggregation and, thus, the water retention capacity. Overall, the BD amended soils were less prone to C losses after tillage. This finding supports the recent indications of absent compromising effects on soil fertility after a regular application of BD since the C fractions, which were already converted into biogas during AD, would otherwise decompose preferably in soils after tillage. This supposed equivalence of the lost C fractions should be monitored both in long- and short-term field studies, for example by tracing with stable isotopes. Nevertheless, following investigations should include fertiliser strategies with undigested organic amendments after tillage to improve knowledge about the effect of BD on C losses after tillage and SOM reproduction.

Pyrolysis-field ionisation mass spectrometry (Py-FIMS) showed that the suggested differences in SOM composition caused by the different soil amendments appeared after just one season with amendment in a maize (*Zea mays* L.) field. After only one single amendment with BD in spring, a lower share of easily decomposable compounds like carbohydrates but a higher share of recalcitrant compounds like lignin were detected in the soil in autumn compared to the mineral fertilised treatment. However, the recalcitrance of lignin-derived substances from anaerobic digestion is questioned since a turnover of these stable leftovers were observed during the days after tillage. This finding is hypothesised to result from the scarcity of easily available C for microorganisms, which let the latter draw on lignin-containing sources instead. Nevertheless, the depletion of carbohydrates due to increased mineralisation after tillage was confirmed for the mineral fertilised treatment.

Directly after the application of BD in concentrations typical for injected application in arable soils, the potential losses of N_2O and N_2 were controlled mainly by gas diffusivity as a function of soil texture and water content. The loamy sand with its relative high share of macropores showed generally low emissions of this gases in aerobic headspace, irrespective of water content, whereas their flux rates and their N_2 / (N_2O+N_2) product ratio increased with water-filled pore space in the clayey silt. The latter soil type is characterised by a relative high share of micropores, which are less favourable for gas diffusion, especially in interaction with soil water. However, high N_2 emission rates after switching the headspace to anaerobic conditions demonstrated that the principally potential for denitrification was also present in the loamy sand.

Doubling the amount of applied BD showed no significant effects on the emitted flux rates of N_2O and N_2, probably due to the inhibitory effect of high NH_3 and NH_4^+ concentrations, respectively, on NO_2^- oxidisers. In combination with the increased pH of BD, the accumulation and subsequent reduction of NO_2^- to nitric oxide (NO), N_2O and N_2 for detoxification is suggested. The measured gaseous N losses of N_2O and N_2 or even the supposed NO losses resulting from such high concentrations of BD question the environmental benefits from reduced NH_3-N emissions after BD injection into soil (compared to surface application followed by immediately homogeneous incorporation).

Overall, the presented results show the need to take account of the special BD characteristics. The changed chemical qualities of BD compared to undigested organic amendments seem to modify the paths of C and N cycling in soils, but the present results do not suggest any seriously varied impacts neither on soil fertility nor on environment. However, the recommendations of best practices for management activities like the application by injection should be checked and adapted to the unique characteristics of BD if applicable. Nevertheless, further short and long-term investigations are necessary to study the impact of BD on soil properties affecting GHG emissions. For an environmental sound agriculture, also the N gas species NO and NH_3 should be taken into account due to their potential for ecosystem acidification. Finally yet importantly, research on the role of N_2 emissions is essential to fully reveal biogeochemical processes responsible for N cycling in agricultural soils, which include the development and advancement of methods for its determination.

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